Vol. XLIV No. 2

February 1955

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

1852 A.Ph.A. 1955 SECOND CENTURY

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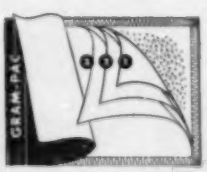

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The Journal of the American Pharmaceutical Association is published monthly by the American Pharmaceutical Association in two editions: Scientific Edition and Practical Pharmacy Edition, under the supervision of the Committee on Publications of the Council of the Association. Publication office: 20th and Northampton Streets, Easton, Pennsylvania.

Editorial and Executive Offices—2215 Constitution Avenue, N.W., Washington 7, D. C. Robert P. Fischelis, Secretary and General Manager.

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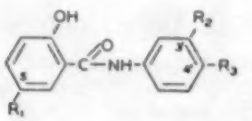
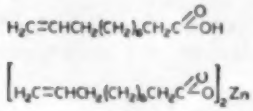
Entered as second class matter January 23, 1917, at the Post Office at Easton, Pennsylvania, under the Act of March 3, 1879, as 24 times a year; Scientific Edition monthly on the 5th: Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

NNR

PRODUCTS RECENTLY ACCEPTED BY THE A. M. A. COUNCIL ON PHARMACY AND CHEMISTRY

ZINCHLORUNDESAL:—Salundek (New) (Wallace & Tiernan).—A mixture containing 2% 5-chlorosalicylanilide, 1% 5,3′-dichlorosalicylanilide, 1% 5,4′-dichlorosalicylanilide, 3% salicylanilide N. F., 2% undecylenic acid-N. F., and 10% zinc undecylenate N. F. Their structural formulas may be represented as follows:





Actions and Uses.—Zinchlorundesal is effective topically in the treatment of tinea capitis caused by Microsporon audouini. Its use generally should be restricted to this purpose because of its potential irritant effects, although zinchlorundesal is also effective in the treatment of superficial dermatomycoses. If a cure is not obtained in four months, X-ray treatment should be considered.

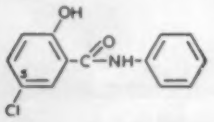
In zinchlorundesal the irritant potentialities of the salicylanilides are minimized because lower concentrations are used, which are nevertheless highly effective when combined with one another and with the relatively nonirritating undecylenic acid components. The fungistatic potency of salicylanilide *in vitro* is approximately eight times that of undecylenic acid against M. audouini, and the chlorosalicylanilides are 5 to 150 times as active as salicylanilide in inhibiting the growth of this microorganism.

Dosage.—Zinchlorundesal is applied topically in the form of an ointment containing the stated proportions of the active ingredients. It is rubbed on the affected and adjacent areas twice daily.

Tests and Standards.—

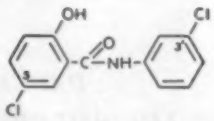
For descriptions and standards for salicylanilide, undecylenic acid, and zinc undecylenate, see the National Formulary under Salicylanilide, Undecylenic Acid, Compound Undecylenic Acid Ointment, and Zinc Undecylenate. For tests and standards for 5-chlorosalicylanilide, 5,3′-dichlorosalicylanilide, and 5,4′-dichlorosalicylanilide, see the monographs for these materials.

5-CHLOROSALICYLANILIDE. — $C_{13}H_{10}ClNO_2$.—M. W. 247.68.—The structural formula of 5-chlorosalicylanilide may be represented as follows:



Physical Properties: 5-Chlorosalicylanilide is a white, odorless, crystalline solid, m.p. 210–213°. It is soluble in alkalis, but only very slightly soluble in acids. The approximate amounts that dissolve at 25° in the following solvents to form 100 ml. of solution are: 2 Gm. in acetone, 0.4 Gm. in alcohol, and 7 Gm. in ether. 5-Chlorosalicylanilide is fairly stable to heat, light, and moisture.

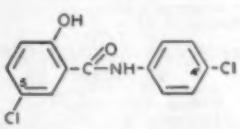
5,3′ - DICHLOROSALICYLANILIDE. — $C_{13}H_9Cl_2NO_2$.—M. W. 282.12.—The structural formula of 5,3′-dichlorosalicylanilide may be represented as follows:



Physical Properties: 5,3′-Dichlorosalicylanilide is a white, odorless powder, m.p. 218–221°. It is soluble in alkalis, but only very slightly soluble in acids. The approximate amounts that dissolve at 25° in the following solvents to form 100 ml. of solution are: 3 Gm. in acetone, 0.3 Gm. in alcohol, and 1 Gm. in ether. 5,3′-Dichlorosalicylanilide is fairly stable to heat, light, and moisture.

[For more information regarding this monograph see *J. Am. Med. Assoc.*, **156,** 1331 (December 4, 1954).]

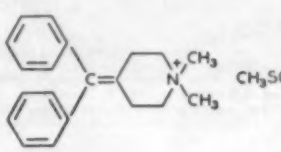
5,4′ - DICHLOROSALICYLANILIDE. — $C_{13}H_9Cl_2NO_2$.—M. W. 282.12.—The structural formula of 5,4-dichlorosalicylanilide may be represented as follows:



Physical Properties: 5,4′-Dichlorosalicylanilide is a white, odorless, crystalline material, m.p. 224–227°. It is soluble in alkalis, but only very slightly soluble in acids. The approximate amounts that dissolve at 25° in the following solvents to form 100 ml. of solution are: 2 Gm. in acetone, 0.4 Gm. in alcohol, and 0.7 Gm. in ether. 5,4′-Dichlorosalicylanilide is fairly stable to heat, light, and moisture.

[For more information regarding this monograph see *J. Am. Med. Assoc.*, **156,** 1331 (December 4, 1954).]

DIPHEMANIL METHYLSULFATE.—Prantal Methylsulfate (Schering).—$C_{20}H_{24}N.CH_3SO_4$.—M.W. 389.50.—4-Diphenylmethylene-1,1-dimethylpiperidinium methylsulfate.—The structural formula of diphemanil methylsulfate may be represented as follows:



Actions and Uses.—Diphemanil methylsulfate is a quaternary parasympatholytic agent that selectively blocks the transmission of nerve impulses through parasympathetic ganglia. At the dosage level required to block parasympathetic ganglia, it does not block sympathetic ganglia. Diphemanil methylsulfate also inhibits gastric secretion and motility and relieves pylorospasm at a lower dosage than that required to inhibit motility of the small and large intestine. A slightly larger dosage effectively blocks cholinergic secretory nerve impulses to the sweat glands. The drug possesses considerable bronchodilator action, but further studies are required to establish its clinical usefulness in bronchial asthma.

Diphemanil methylsulfate is useful as an adjunct in the treatment of peptic ulcer, gastric hyperacidity, and hypermotility as in chronic hypertrophic gastritis, certain less specific forms of gastritis, and pylorospasm. It is not proposed for the control of spasm or hypermotility of the intestinal and urinary tracts. The drug is effective for the treatment of hyperhidrosis and also for the control of sweating when this aggravates certain dermatoses.

Diphemanil methylsulfate is not readily absorbed from the gastrointestinal tract nor reabsorbed from the vascular system into the gastrointestinal tract. Absorption by the oral route is reduced by the presence of foods, antacids, or bile salts in the stomach, but such interference largely can be obviated if the drug is administered between meals. Following parenteral injection, about 50% of the drug is excreted unchanged, chiefly in the urine; the remaining 50% has not been accounted for, although no evidence of storage has been found.

At its anticholinergic dose level of therapeutic action, diphemanil methylsulfate may be associated with atropine-like side-effects, including xerostomia, mydriasis, tachycardia, constipation or diarrhea, and urinary retention. Such reactions usually are minimal, but they may interfere with therapy in some patients. As with other parasympathetic blocking agents, the drug usually is contraindicated in patients with glaucoma. An injectable solution of physostigmine methylsulfate may be administered in the usual adult dose of 2 mg. subcutaneously as an antidote to counteract the antiacetylcholine activity of diphemanil methylsulfate.

Dosage.—Diphemanil methylsulfate is administered orally and by subcutaneous or intramuscular injection. For the management of peptic ulcer, pylorospasm, and gastric hyperacidity and motility in hypertrophic and other forms of gastritis, the drug may be injected for initial control of acute symptoms or management of acute episodes. The usual adult oral dosage for initial treatment or after injection to control acute symptoms is 100 mg. every four to six hours (between meals) prescribed as ordinary tablets. This dosage should be increased or decreased in accordance with the response; a dosage of 150 to 200 mg. may be required in some patients. Maintenance dosage usually may be reduced to 50 to 100 mg. Oral therapy also may be prescribed in the form of a coated tablet that prolongs the action of the drug over a period of eight hours. In such form, 100 mg. administered at eight hour intervals usually is adequate, but this may be increased to 200 mg. every eight hours if necessary to maintain control of symptoms.

When injected for initial control of symptoms or acute episodes, the usual dosage is 0.5 mg. per Kg. of body weight administered subcutaneously or intramuscularly four times daily. A parenterally administered dose of 50 mg. should not be exceeded except with extreme caution. Injection of the drug preferably should be continued for 24 to 48 hours after symptoms are brought under control; thereafter, therapy should be continued by the oral route.

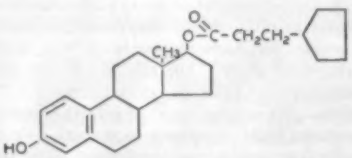
For the treatment of hyperhidrosis or control of sweating aggravating dermatoses, the usual oral dosage for adults is 100 to 200 mg. three times daily (between meals) prescribed either as ordinary or prolonged-acting tablets. Following inhibition, decreased dosage may be adequate to prevent recurrence.

Tests and Standards.—

Physical Properties: Diphemanil methylsulfate is a white or near white, bitter, crystalline solid with a faint characteristic odor, m.p. 189–196°. It is very slightly soluble in ether. The approximate amounts that dissolve at 25° in the following solvents to form 100 ml. of solution are: 3 Gm. in alcohol, 3 Gm. in chloroform, and 3 Gm. in water. Diphemanil methylsulfate is stable to heat and light but is somewhat hygroscopic. The *p*H of a 1% solution is 4.0–6.0.

[For more information regarding this monograph see *J. Am. Med. Assoc.*, **157**, 342 (January 22, 1955).]

ESTRADIOL CYCLOPENTYLPROPIONATE.—$C_{26}H_{36}O_3$.—M.W. 396.55.—3-Hydroxy-Δ-1,3,5-estratriene-17-cyclopentylpropionate.—The structural formula of estradiol cyclopentylpropionate may be represented as follows:



Actions and Uses.—Estradiol cyclopentylpropionate has the same actions and uses as estradiol and its other fat-soluble esters. (See New and Nonofficial Remedies under the monographs on estradiol, estradiol benzoate, and estradiol dipropionate.) In vegetable oil solutions for intramuscular injection, estradiol cyclopentylpropionate may produce more prolonged estrogenic effects than similar oil solutions of either estradiol benzoate or estradiol dipropionate. In women in the menopause, the average duration of estrogenic action, as measured by vaginal smear, is approximately three to four weeks after a single injection of 5 mg. in oil. Relief of vasomotor symptoms appears within one to five days and is maintained one to eight weeks.

Estradiol cyclopentylpropionate is not associated with adverse reactions to any greater extent than may be encountered with injectable oil solutions of other esters of estradiol. It should be employed with the same precautions as with the administration of similar preparations and of estrogens in general.

Dosage.—Estradiol cyclopentylpropionate is administered in oil solutions by intramuscular injection only. Initially, a single dose of 1 to 5 mg. is injected weekly for two or three weeks; for maintenance the dosage interval may be lengthened to three to four weeks.

Tests and Standards.—

Physical Properties: Estradiol cyclopentylpropionate is a white, odorless, crystalline solid, m.p. 148–152°. It is freely soluble in chloroform and in ether, and practically insoluble in water and in alkalies. The approximate amounts that dissolve at 25° in the following solvents to form 100 ml. of solution are: 2 Gm. in alcohol and 2 Gm. in methanol.

[For more information regarding this monograph see *J. Am. Med. Assoc.*, **157**, 344(January 22, 1955).]

OXYTETRACYCLINE-POLYMYXIN B.—Terramycin Hydrochloride with Polymyxin B Sulfate (Pfizer).

Actions and Uses.—Oxytetracycline-polymyxin B, a mixture of oxytetracycline hydrochloride and polymyxin B sulfate, is useful for ophthalmic application in the prevention and treatment of pyogenic mixed surface infections of the eye that are likely to be susceptible to either or both of these antibiotics. Because polymyxin B is considered the antibiotic of choice against pseudomonal infections, its use in fixed combination with a broad spectrum antibiotic may be justified on the basis that the incidence of ocular infections complicated by the presence of *Pseudomonas aeruginosa* apparently is increasing. The particular effectiveness of polymyxin B against gram-negative bacteria enhances the action of oxytetracycline against both gram-positive and gram-negative organisms. While synergism rarely may be demonstrable against certain types of infection, the actions of the components should be regarded as primarily additive; therefore, the proportions should correspond to the usual concentrations in which each is employed singly.

Oxytetracycline-polymyxin B is considered effective in the treatment of acute and subacute purulent conjunctivitis, acute catarrhal conjunctivitis, and chronic blepharoconjunctivitis not involving the meibomian gland; the mixture is also effective as a prophylactic prior to ocular surgery. It may be useful in the management of infection complicating a corneal ulcer, epiphora secondary to conjunctival infection, and acute trachoma. The mixture should not be employed when bacteriological studies indicate that a specific ocular infection is more susceptible to a single antibiotic or to another antibacterial agent. If the mixture is used at all in the treatment of gonococcic conjunctivitis, orbital cellulitis, keratitis, uveitis, retinitis, and other deep-seated infections, it should be supplemented by systemic antibiotic or other therapy that is indicated.

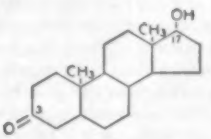
Oxytetracycline-polymyxin B usually is well tolerated by the membranes of the eye. Allergic reactions may be encountered but are rare. If severe reactions occur, use of the mixture should be discontinued. When sensitization to only one component occurs, therapy with the other alone may be continued if the infection is susceptible to it. Bacterial resistance to either antibiotic component ordinarily does not develop, even under continuous therapy.

Dosage.—Oxytetracycline-polymyxin B is applied only topically to the eye as an ophthalmic ointment containing the equivalent of 5 mg. of oxytetracycline base and 10,000 units of polymyxin B base per Gm. For the treatment of surface ocular infections, a small quantity of such ointment is applied to each affected eye four to six times daily; for prophylaxis in operative procedures, a small quantity is placed in both eyes several times during the day preceding surgery and into the operated eye at the time of each dressing. In blepharitis, scales and crusts should be removed and the ointment applied over the lid margin. Treatment lasting from 10 to 14 days is usually sufficient for acute infections; in chronic cases, treatment may be required up to three months. Careful clinical follow-up is advisable. If infection persists, a second course or change of treatment should be instituted.

Tests and Standards.—

Oxytetracycline hydrochloride and polymyxin B sulfate are described in their respective monographs in Tests and Standards for New and Nonofficial Remedies.

STANOLONE.—Neodrol (Pfizer).—$C_{19}H_{30}O_2$.—M. W. 290.43.—Androstane - 17(β) - ol - 3 - one.—The structural formula of stanolone may be represented as follows:



Actions and Uses.—Stanolone is an androgen that has the same actions and uses as testosterone and its esters. (See New and Nonofficial Remedies under the general statement on testes.) It is useful clinically for its anabolic and tumor-suppressing actions in selected cases of inoperable carcinoma of the breast or postoperative metastatic carcinoma of the breast. Its use, which must be weighed against its inherent virilizing and metabolic effects, should be subject to the same precautions and contraindications as is the use of other androgenic agents.

Dosage.—Stanolone is administered by intramuscular injection. Like free testosterone, an aqueous suspension of microcrystalline stanolone should be expected to produce a slightly less intense and slightly more prolonged androgenic action than an equivalent oil solution of its propionic acid ester. In carcinoma of the breast, the average effective dosage is 100 mg. daily. This dosage should be continued as long as the patient shows improvement or until the patient is unable to tolerate androgenic therapy because of severe virilization or untoward metabolic effects. Lower dosage may be better tolerated but is considered ineffective against carcinoma of the breast. The dosage for the treatment of testicular hormone defficiency, functional uterine bleeding, or postpartum suppression of breast engorgement remains to be established by experience with its use in these conditions.

Tests and Standards.—

Physical Properties: Stanolone is a white, odorless, crystalline powder, m.p. 175–183°. It is practically insoluble in water. The approximate amounts that dissolve at 25° in the following solvents to form 100 ml. of solution are: 6 Gm. in alcohol and 1.5 Gm. in ether.

[For more information regarding this monograph see *J. Am. Med. Assoc.* **157**, 345(January 22, 1955).]

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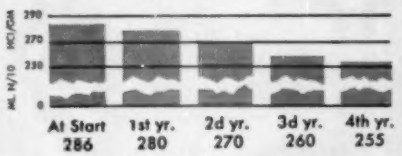

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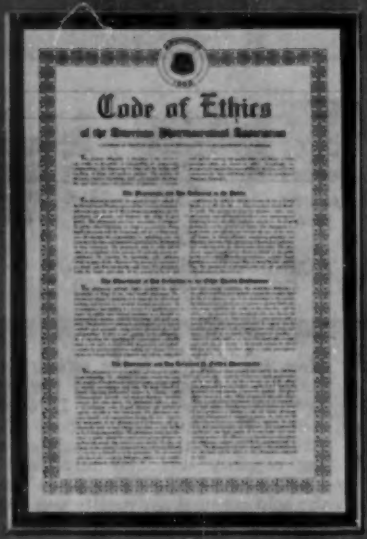

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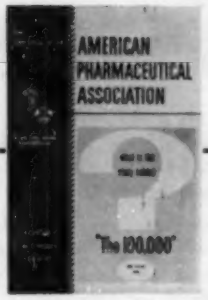

A Preview of Advertising

In the February 1955 issue of the Practical Pharmacy Edition

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLIV — *FEBRUARY, 1955* — NUMBER 2 — CONSECUTIVE NO. 4

The Metabolism of *p*-Aminosalicylic Acid (PAS) in Man*

By E. LEONG WAY, CHIN-TZU PENG, NASEEM ALLAWALA and T. C. DANIELS

The urinary excretion of *p*-aminosalicylic acid (PAS) was studied in five individuals after single and repeated dosage. Making use of countercurrent distribution and filter paper chromatography, three urinary products were isolated and characterized and several others were characterized but not isolated. The compounds isolated and characterized represented the following percentiles of the total dosage administered: unchanged PAS (14–33 per cent); acetyl *p*-aminosalicylic acid (28–63 per cent); *p*-aminosalicyluric acid (0–26 per cent); possibly two unknown free amines (2–10 per cent); and one unknown bound amine (3–10 per cent).

DESPITE THE WIDE USAGE of *p*-amino salicylic acid (PAS) in the treatment of tuberculosis much remains to be learned concerning the metabolism of this compound. An earlier study (1) on the urine of an individual taking PAS revealed, after countercurrent distribution extraction, the presence of three free amines and two conjugated amines. Two of the three amines were identified as unchanged PAS and its glycine conjugate, *p*-aminosalicyluric acid (PASU) and one of the conjugated amines was acetylated *p*-aminosalicylic acid (AcPAS). The identifications were based chiefly on the partition behavior of the compounds without isolation. These studies have been extended and the present communication describes the isolation of PASU and AcPAS from the urine of man and gives evidence for some additional metabolites of PAS.

* Received August 27, 1954, from the College of Pharmacy and the Department of Pharmacology, School of Medicine, University of California, San Francisco, Calif.

This study was supported by the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service. The elemental analyses of PASU and AcPAS were carried out by the Microanalytical Division, University of California, Berkeley; the MAP analysis by the Pharmaceutical Department, Calco Chemical Division, American Cyanamid Co. The urine specimens were obtained through the courtesy of the University of California Tuberculosis Ward at the San Francisco County Hospital. The PAS used in these studies was donated by Barnes-Hind Pharmacy. We are grateful to Dr. Eugene Jorgenson and Miss Bertha Huang for their cooperation. The PASU was furnished by Dr. H. B. Lewis who obtained the compound from Dr. Leonard Doub of Parke, Davis & Company.

EXPERIMENTAL PROCEDURES

Estimation of Free Amine.—Free amines in urine and blood were determined spectrophotometrically according to the method previously published (1). Color development involves diazotization of the amine at 0° and subsequent complexing with N-(1-naphthyl)ethylenediamine dihydrochloride in the presence of acetic acid.

Estimation of Conjugated Amine.—The above procedure was followed after hydrolyzing the sample to be tested in the manner previously described [incubation for eighteen hours at 37° in the presence of 2.4 *N* HCl (1)]. The total amines thus determined minus the free amines give the conjugated amines.

Separation and Characterization of Free and Conjugated Amines in Urine.—Countercurrent distribution and paper chromatography were employed. Total urine was collected over a twenty-four hour period from five individuals on varying dosage regimens of PAS and analyzed for free amine and total amine.

For the countercurrent studies a 20-cc. aliquot of the urine was brought to *p*H 3.59 by addition of

2 *M* acetate buffer and extracted repeatedly with ether. The pooled ether extracts (approximately 1 liter) were evaporated to dryness at room temperature under reduced pressure and the resulting residue taken up in 20 ml. of *p*H 3.44 acetate buffer. In later samples the urine was lyophilized to a dry powder which was then dissolved in 2 *M* acetate buffer, *p*H 3.44. A 7.8-cc. aliquot was subjected to a twenty-four transfer separation in a countercurrent distribution apparatus using as the solvent pair the same buffer and isoamyl alcohol. The upper isoamyl alcohol phase was moved. After completion of the distribution procedure both layers in each tube were analyzed for free amine and total amine. The amines in the isoamyl alcohol layer were transferred to water solution by extraction with 0.1 *N* NaOH before analysis. The experimental curves were fitted according to the method of Way and Bennett (2).

Parallel to the countercurrent distribution studies, paper chromatography was employed on the urine of the same five individuals plus at least ten other individuals for the purpose of correlating and confirming the findings on the presence of various metabolites of PAS. The original urine samples were usually studied but in one experiment chromatograms were developed on the urinary fractions obtained after countercurrent distribution. The descending technique was adopted, using approximately 0.05-ml. aliquots of each sample on strips of Whatman No. 1 filter paper. As a general rule the unknown samples were run alongside of known compounds. Several strips of a particular sample were run simultaneously to allow for color-development by different procedures.

After testing various solvents, two solvent systems were found to yield satisfactory resolution of the PAS metabolites. One system consisted of a mixture of five parts of dioxane and 1 part of water (CS II) and the other a mixture of *n*-butanol, ethyl alcohol and 3 *N* NH_4OH in the proportion of 4:1:5, all by volume (CS I). With the latter solvent system more reproducible results were obtained by equilibrating the loaded paper with the solvent in the chromatography cabinet for twelve hours before irrigation. The irrigated paper was dried and developed, using Ehrlich reagent for free amines and ferric nitrate (1% in 0.07 HNO_3) for free phenols.

RESULTS

Both the countercurrent and the filter paper chromatographic experiments indicated the presence of several biotransformation products of PAS in the urine following its administration. Further correlation of the two separation procedures was obtained by chromatographing all 24 urine fractions of patient A. C. obtained after countercurrent distribution on paper. From these studies the identity of four compounds was established: PAS, AcPAS, PASU and *m*-aminophenol (MAP), and three or four other metabolic products, X_1, X_2, X_3 and X_4? have been partially characterized but not identified.

The countercurrent experiments indicated the presence of five substances. As an example of these studies the experimental curve following countercurrent distribution of the urine of patient A. C. is shown in Fig. 1. In confirmation of earlier findings, the curve shows three maxima for free amines and two maxima for bound amines. Each major peak corresponds to the following: tube 1, an unknown free amine, X_1; tube 4, an unknown bound amine, X_2; tube 11, PASU; tube 19, PAS and AcPAS. The countercurrent studies are summarized in Table I, which also gives the percentage of the PAS dose accounted for in the urine and a breakdown of relative calculated contribution of each metabolite.

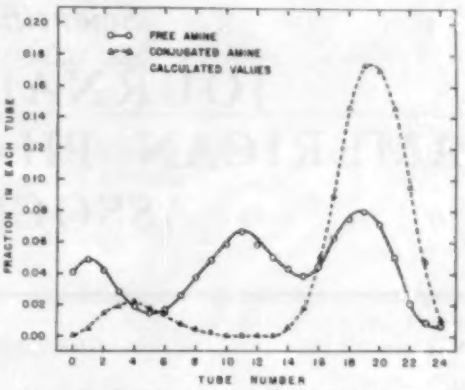

Fig. 1.—Countercurrent distribution curves of free and conjugated amines in the urine of A. C. after taking PAS.

The paper chromatographic studies usually indicated the presence of six and occasionally seven substances. The excreted products of PAS tended to distribute themselves with increasing R_f values as follows: PASU, X_1, PAS, X_4?, AcPAS, X_2, MAP. Among these, PASU, X_1, PAS, X_4?, and MAP gave yellow spots when sprayed with Ehrlich reagent, whereas PASU, PAS, AcPAS and X_2 gave violet color with ferric nitrate. The range of R_f values obtained for each compound with each solvent is shown in Fig. 2.

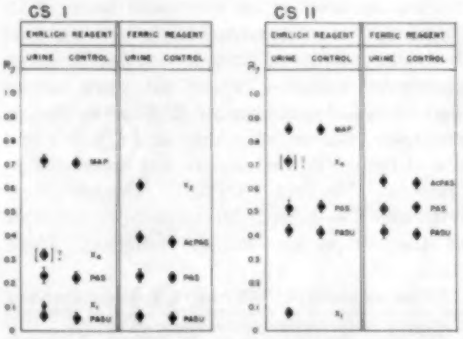

Fig. 2.—Schematic representation summarizing paper chromatography results obtained on urines from individuals on PAS.

Correlation of the countercurrent with the paper chromatographic studies on the urine of A. C. is shown schematically in Fig. 3. The compounds which were detected are placed in relative order of increasing R_f on the ordinate, and the bar indicates

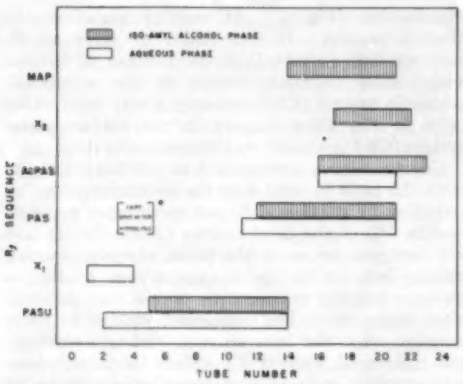

Fig. 3.—Schematic chart correlating countercurrent distribution and paper chromatography results on the urine of A. C.

qualitatively where a positive test was obtained for each compound on the separated urinary fractions contained in each tube. The intensity of the spots generally correlated well with the concentration in each tube. The results on all the studies are treated in greater detail under the heading for each compound.

p-Aminosalicylic Acid (PAS).—This corresponds to the free amine in the experimental countercurrent distribution curve with the maximum in tube 19 (Fig. 1). A calculated curve for a substance with a partition ratio (K) of 3.40, comprising 40% of the total free amines yielded a reasonable fit to the experimental values. As found previously (1) the calculated K was within limits of experimental error to the K obtained with a known sample of PAS in the same solvent pair.

Additional evidence as to the presence of unchanged PAS in the urine is indicated by the results using paper chromatography. The R_f values of the material in tubes 11–21, as well as of the original urine sample, were found to agree closely with those of PAS added to urine. The R_f values of added PAS in each solvent system were between 0.20–0.24 in CS I and 0.49–0.52 in CS II; those of the recovered material were, respectively, 0.20–0.22 in CS I and 0.50–0.53 in CS II. Chromatograms on the original urine samples of the other individuals, also indicated the presence of PAS. As shown in Fig. 2 the range of the R_f values is in good agreement with that of added PAS.

The countercurrent studies indicate that the amount of PAS excreted by five individuals was fairly consistent despite the various dosage regimens, the excretion range being 17 to less than 33 per cent (calculated from Table I).

N-Acetyl-p-aminosalicylic Acid (AcPAS).—The conjugated amine with a peak in tube 19, representing 90 per cent of the total bound amines, yielded a calculated partition ratio closely approximating that of authentic AcPAS. The recovered material in tubes 16–23 was also found to have, within limits of experimental error, identical R_f values with those of AcPAS. Filter paper chromatograms on the original urine specimens of the other individuals also revealed the presence of AcPAS (Fig. 2).

AcPAS was also isolated in crystalline form by conventional methods. The urine of C. T. P. (100 cc.) was extracted with several portions of ether and ether extracts pooled and evaporated to dryness under reduced pressure. The residue was treated with 1 N hydrochloric acid to solubilize the free amines and then filtered. The residue was then dissolved by treatment with a solution of sodium bicarbonate and precipitated by addition of 1 N HCl. The AcPAS thus obtained was recrystallized twice from dioxane, m. p. 230–231.5° (dec., uncorr.).

No change in the melting point was noted when the material was mixed with synthetic AcPAS. The ultraviolet absorption spectrum of the recovered AcPAS was identical to the known compound, exhibiting maxima at 263 mμ and 300 mμ. Upon elemental analysis the following results were obtained:

Anal. Calcd. for $C_9H_9NO_4$: C, 55.37; H, 4.75; N, 7.15. Found: C, 55.27; H, 4.95; N, 7.15.

The countercurrent studies, as summarized in Table I, indicate that the major fraction of conjugated amines appearing in the urine is chiefly represented by AcPAS; moreover, AcPAS represents the chief metabolite derived from PAS.

p-Aminosalicyluric Acid (PASU).—The compound was identified by three separate procedures. In the countercurrent studies the free amine with the maximum in tube 11 was calculated to represent 41% of the total free amines present with a K of 0.89 (Fig. 1). The K obtained with a known sample of PASU using the same solvent pair was found to be 0.92. The identity of the substance as PASU in tubes 2–14 as well as in other original urine specimens was further confirmed by the paper chromatographic studies, Fig. 2 and 3. In addition PASU was isolated by pooling the fractions in the isoamyl alcohol layer of tubes 7–14. The isoamyl alcohol was removed under reduced pressure and the resulting residue taken up in ether. On bubbling hydrogen chloride through the ether solution, the

TABLE I.—THE METABOLIC FATE OF p-AMINOSALICYLIC ACID (PAS) AS DETERMINED BY COUNTERCURRENT DISTRIBUTION

Patient	NaPAS Regimen	Per Cent of 24 Hour Dose Accounted for	Per Cent of Total Amines as: PAS	PASU	X_1 (free)	AcPAS	X_2 (bound)
D. L. H.*	5 Gm. 2X for 1 day	81	21	15	4	54	6
E. L. W.	5 Gm. 3X for 1 day	73	24	18	3	50	5
M. J. F.	5 Gm. 3X for 1 day	87	21	29	6	37	7
C. T. P.	5 Gm. 3X for 1 day	84	25	0	10	60	5
A. C.	5 Gm. 3X for 140 days	..	24	25	11	38	2
J. G.	5 Gm. 3X for 320 days	..	33	26	10	28	3

* Data from Way, *et al.* (1).

hydrochloride of PASU was precipitated, separated by filtration, and purified by recrystallization from absolute alcohol, m. p. 214–215° (dec., uncorr.); mixed m. p. with synthetic hydrochloride of PASU, [prepared by Doub, *et al.* (5)] 214–215°. Ultraviolet absorption studies failed to reveal any differences between recovered and synthetic PASU.

Elemental analyses of recovered products yielded the following: Calcd. for $C_9H_{10}N_2O_4HCl$: C, 43.8; H, 4.5; N, 11.4. Found: C, 44.0; H, 4.4; N, 11.4.

PASU was also isolated from the dried ether extract of urine (M. J. F.) without carrying through the countercurrent distribution. The residue from the extract was taken up in 0.5 *M* acetate buffer *p*H 3.59 and extracted twice with ten times its volume of 2% ether in benzene to remove PAS. The buffer layer was then decolorized with charcoal and extracted with large volumes of ether. The ether was evaporated to a small volume and saturated with hydrogen chloride. The resulting precipitate of PASU hydrochloride was separated and dried; mixed m. p. with synthetic PASU, 215° (dec., uncorr.).

A wide excretion range (0– <33%) was obtained for PASU primarily because one of the individuals (C. T. P.) failed to excrete the compound. In this instance both the paper chromatographic and countercurrent methods indicated the absence of PASU.

***m*-Aminophenol (MAP).**—The presence of MAP was revealed in the paper chromatography studies by Ehrlich reagent but not by ferric nitrate. A spot corresponding to MAP was obtained on most urine specimens which were studied after being kept in a refrigerator overnight and in tubes 14–21 of the urine of A. C. (Fig. 3). However, when tests were conducted on fresh urine collected within a few hours after the first PAS dose, a spot for MAP failed to appear although positive tests were obtained for PAS, AcPAS, and PASU. When the urine was allowed to stand for more than one hour at room temperature, a positive test for MAP was usually obtained. Since MAP readily yields a positive test for an aromatic amine, the failure to detect its presence by countercurrent distribution indicates that only minor amounts were present. Any appreciable amounts would affect the PAS peak in region of tube 19 since the partition ratio of MAP in the solvent system was found to be near that of PAS.

Unknown Metabolites.—Three or four metabolites of PAS have been partially characterized but not identified.

Compound X_1 was detected in fresh urine specimens collected after PAS therapy and corresponds to the free amine with the peak in tube 1 of the countercurrent distribution (Fig. 1) and with the R_f values 0.12 in CS I and 0.08 in CS II by paper chromatography (Fig. 2 and 3). It usually constituted less than 10% of the total amines. It yielded an intense yellow spot with Ehrlich reagent when the irrigation solvent was dioxane and water (CS II) but only a faint yellow coloration in the *n*-butanol-ammonia system (CS I). It failed to give a test with the iron reagent.

Compound X_2, possibly a phenolic substance, was present in virtually all the original urine samples collected after PAS therapy and in the isoamyl alcohol layer of tubes 17-21 in the countercurrent distribution (Fig. 3). It reacted negatively to Ehrlich reagent. It was found to have an R_f between 0.60 to 0.64 (Fig. 2), yielding an intense violet color to ferric nitrate in the *n*-butanol-ammonia system (CS I) but only a very faint violet color to the same reagent in the dioxane-water system (CS II) when it was occassionally detected.

Compound X_3, corresponds to the bound amine with the peak in tube 4 of the countercurrent distribution (Figs. 1 and 3) and constitutes approximately 5% of the total amines (Table I). It was not detected *per se* in the paper chromatographic studies but, as in the countercurrent studies, a positive test for an aromatic amine was obtained after hydrolysis. The compound yielded no color reaction with the iron reagent. After hydrolysis the compound yielded R_f values by paper chromatographic analysis corresponding to those of PAS; however, only a faint positive test was obtained with the Ehrlich reagent.

Compound X_4? was not detected in the countercurrent studies but was revealed by paper chromatography in several but not all original urine specimens collected after PAS administration. It was not found in the control urine of several individuals who received no PAS. It yielded an intense yellow with the Ehrlich reagent and R_f values of approximately 0.32 in CS I and 0.72 in CS II (Fig. 2).

DISCUSSION

Studies on the fate of PAS in various species have revealed considerable differences in the metabolic pathways traversed although some routes are in common. This is not surprising since PAS possesses three functional groups (—COOH, —NH_2, and —OH) in addition to the aromatic ring which conceivably can undergo biotransformation. Apparently most if not all species excrete PAS chiefly in the urine as the unchanged compound and metabolic products with the average recovery in man following oral administration being in the neighborhood of 85% of the dose.

With the possible exception of the dog (1), all species appear to excrete a considerable fraction of the PAS dosage as a conjugated amine, presumably AcPAS. Isolation of crystalline AcPAS from the urine of rabbits has been reported by Venkataraman *et al.* (3), and the present communication confirms these findings in man. In man AcPAS constitutes by far the major portion of the bound amines present in the urine. In fact AcPAS appears to be the major metabolite of PAS, the range being less than 28–48%. Somewhat similar percentages were reported by Lehman (4), who gave values between 25–35%. The formation of AcPAS appears to be of considerable pharmacologic importance according to Bertolani (6) who has reported that the analgesic, antipyretic, and antirheumatic properties of PAS are mainly linked to its formation of AcPAS.

To the best of our knowledge, PASU has not been previously isolated as a urinary metabolite of PAS. There has been some earlier indication that PASU may be a PAS metabolite (1, 4) in man. Earlier work on other species has not always given affirmative results. With the exception of the aforementioned works (1, 4) none of the papers on the fate of PAS have any data pertaining to PASU.

Venkataraman, *et al.*, looked for but found no evidence for glycine conjugation with PAS in the rabbit (3). Evidence that such a reaction may occur in the rat and the dog was suggested from the fact that the partition behavior of the free urinary amine (s) present following PAS differed from that of the parent compound (1). More concrete evidence was obtained on the urine of one individual using countercurrent distribution techniques (1). These studies have since been confirmed by Lehman (4) by paper chromatography. The present investigation furnishes conclusive evidence that in man PAS is conjugated with glycine to PASU and excreted in the urine. In agreement with Lehman our preliminary studies indicate that the compound may also be present in blood in low but significant amounts and, therefore, may interfere with blood PAS determinations.

The studies also indicate that PAS conjugation with glycine is a major metabolic pathway in most individuals. Lehman (4), using semiquantitative methods, reported values for FASU from urines of patients on continuous PAS therapy to be between 28–50% as compared to our present range from 0 to less than 26%.

Although the results could not be quantitated, the *m*-aminophenol (MAP) found in urine was proportionately greater than in the original PAS samples. Of the two lots of PAS used, 0.02% MAP was found to be present in one sample, none in the other. When samples of both lots were diluted to make standard solutions of PAS and chromatographed, no test for MAP was revealed by the Ehrlich reagent on the freshly prepared standards. However, the presence of MAP in the urine does not necessarily mean that it is a true metabolite of PAS. In aqueous solution PAS is easily decarboxylated to *m*-aminophenol (3) and conceivably this process may occur *in vivo* without participating in enzymatic processes. We have found that PAS readily decarboxylates when incubated with human blood at 38° at a considerably greater rate (about one-half in twenty hours) than can be accounted for *in vivo*. The failure to find larger amounts of MAP excreted may be explained in part by the fact that the compound itself may undergo biotransformation. It remains to be studied whether some of the trace amounts of unknown metabolites detected from our studies are formed from MAP.

Lehman (4) has also reported the presence in urine of two unknown free amine metabolites of PAS. These compounds may correspond to compounds X_1 and X_4? in our studies but further studies are necessary to clarify these findings.

SUMMARY

1. A quantitative investigation on the various metabolites of *p*-aminosalicylic acid excreted in the urine of human subjects has been carried out using countercurrent distribution and paper chromatography.

2. *p*-Aminosalicyluric acid and *p*-acetylaminosalicylic acid were found to constitute the chief biotransformation products of *p*-aminosalicylic acid. Both metabolites were isolated in the crystalline state.

3. In addition, *m*-aminophenol and four other unknown products were detected in trace amounts.

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Studies on the Absorption and Excretion of a New Cholecystographic Agent, Teridax®*

By PRESTON L. PERLMAN, ROBERT E. KOSINSKI, and DAVID SUTTER

Information is presented on the mode and rate of absorption, excretion, and blood levels of the cholecystographic agent, Teridax®, following oral administration to man. Data on absorption and excretion are also presented for the dog and rat. In addition, certain comparative data for iodopanoic acid (Telepaque®) are given.

TERIDAX®[1] [triiodoethanoic acid, α-ethyl-β-(2,4,6 - triiodo-3-hydroxyphenyl)-propionic acid] has recently been introduced as a cholecystographic agent and it was deemed of importance to carry out certain studies on the biological disposition of this substance following oral administration.

In this investigation information on the absorption, blood levels (including plasma bound iodine), and excretion following oral administration to man are presented along with comparable data on another cholecystographic agent, Telepaque® [α-ethyl-β-(2,4,6-triiodo-3-aminophenyl)-propionic acid, iodopanoic acid]. These comparative data are of interest, as will be demonstrated, because, although the two compounds differ structurally to only a slight degree, the influence on the metabolic fate of this small differ-

* Received November 19, 1954, from the Biological Laboratories, Schering Corporation, Bloomfield, N. J.
[1] Registered trade mark of Schering Corporation, Bloomfield, N. J.

ence is marked. In addition, absorption and excretion data for the dog and rat are presented for both compounds.

With reference to iodopanoic acid excretion, Shehadi (1), without presenting details, states that "Telepaque is excreted mostly through the intestinal tract. Up to 85 per cent of the total ingested was recovered from the feces of experimental animals, and from 2.1 to 4.9 per cent was excreted in the urine. Traces were found in the internal organs, mostly in the liver." The species of experimental animals referrred to by this investigator was not indicated. Schroder and Rooney (2) reported excretion studies on five subjects and state that "86%...was eliminated in 5 days, 12.4% by way of the urine, and 73.6% by way of the feces...."

METHODS

The method for the estimation of Teridax® in urine, feces, and blood is that of Bang and Georg (3). This procedure, with certain minor modifications was also employed for the estimation of Teridax® in tissues. For the quantitative measurement of plasma bound iodine (PBI), the method of Connor, *et al.* (4), was used.

Recoveries of Teridax® from blood, urine, feces, and tissue (liver and muscle) were carried out with the following average results: blood (95%), urine (98%), stools (103%), liver and muscle (95%). These data are in fair agreement with those previously reported by Bang and Georg for Priodax® (3).

In other experiments, iodopanoic acid recovery with the same method gave results comparable to those obtained with Teridax®.

RESULTS AND DISCUSSION

Urine and Fecal Elimination of Teridax® and Iodopanoic Acid in Human Subjects.[1]—A single three-gram dose of Teridax® was administered to each of four normal male adults following a two-day control period, and aliquots of consecutive total twenty-four-hour urine and stools were collected and analyzed for iodine. From Fig. 1 it will be noted that only a small percentage of the administered dose was eliminated in the stools with the major amount (17–23%) being recovered from the urine during the first forty-eight hours. Despite continued collection of urine (nineteen days) and stools (five days), an over-all recovery of only 25% was realized. Hence, approximately 75% of the dose remained unaccounted for. What role tissue-storage might play in this unaccounted iodine was studied in four dogs and is reported below.

In another experiment a single three-gram dose of iodopanoic acid was administered to each of the four normal male adults, and aliquots of consecutive total twenty-four-hour urine and stools

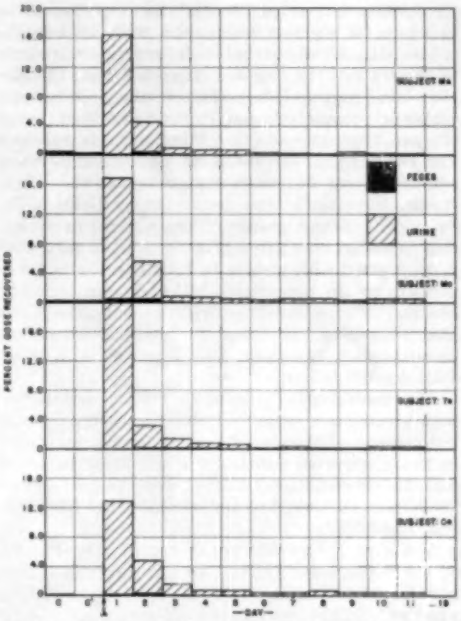

Fig. 1.—Total excretion of Teridax® by four normal persons following an oral dose of 3 Gm.

were collected and analyzed for iodine following a two-day control period. Only a small percentage of the administered dose was eliminated in the urine with the major portion (22–29%), in three of the four subjects, being eliminated in the feces (Fig. 2). After these experiments were completed, the paper by Schroder and Rooney (2) appeared; and because of the differences in results from our data, further studies on Teridax® and iodopanoic acid excretion in human subjects were carried out. It was thought that perhaps fasting or a high fat meal might account for the discrepant results. Accordingly, studies were carried out on fasting subjects, subjects receiving drug according to routine preroentenographic procedure, and subjects consuming the drug during a meal containing a high fat content. The results are given in Table I. If these data are compared, it will be noted that the magnitudes of over-all excretion for both compounds are about the same; the only difference appears to be in the major route of excretion. With Teridax®, of the amount excreted, the greater percentage occurs in the urine; with iodopanoic acid of the eliminated iodine, most is found in the stools. This difference in route of excretion is most certainly a reflection of the small difference in chemical structure between the two compounds. Also, the apparent influence of diet regime on drug excretion should be noted. It would appear that fasting is associated with greater drug retention.

Blood Levels of Teridax® and Iodopanoic Acid in Human Subjects.—Blood levels of Teridax were determined for the first group of four subjects who had received a single 3.0-Gm. dose orally and during the same time that urine and feces were analyzed for iodine. The blood level (Fig. 3) rose fairly rapidly to a maximum at six to eight hours (21–34.2 mg.

[1] We wish to acknowledge the assistance and cooperation of F. M. Offenkrants, M.D., Clinical Pathologist, St. Joseph's Hospital, Paterson, N. J., in making certain of the human subjects available for this study.

TABLE I.—EXCRETION OF TERIDAX® AND IODOPANOIC ACID IN HUMAN SUBJECTS

Drug	Dose (Gm.)	Regimen	Subject	% Drug Excreted (4-day period)[a] Urine	Feces	Total
Teridax®	3.0	Fasting[b]	Mo	28.0	1.1	29.1
	3.0		Ma	24.9	0.9	25.8
	3.0		Cr	21.2	0.2	21.4
	3.0		Tr	26.7	0.2	26.9
			Av.	25.2%	0.6%	25.8%
	3.0	Standard[c]	CJ	39.2	0.8	40.0
	3.0		WB	36.8	0.6	37.4
	3.0		SL	36.0	0.4	36.4
	3.0		TT	36.7	0.6	37.3
	3.0		CC	40.5	0.7	41.2
			Av.	37.8%	0.6%	38.6%
	3.0	Fatty Meal[d]	TE	35.3	0.8	36.1
	3.0		TEw	31.7	0.8	32.5
	3.0		JA	53.2	0.4	53.6
	3.0		JAw	41.5	0.9	42.4
			Av.	40.4%	0.7%	41.2%
Iodopanoic Acid	3.0	Fasting[b]	Ma	4.7	30.3	35.0
	3.0		Mo	3.1	21.7	24.8
	3.0		Tr	5.4	20.9	26.3
	3.0		Na	9.0	7.9	16.9
			Av.	5.5%	20.2%	26.9%
	3.0	Standard[c]	AN	4.1	38.7	42.8
	3.0		SC	18.0	27.7	45.7
	3.0		FW	8.8	28.3	37.1
	2.0		NS	7.1	40.2	47.3
	2.0		HH	13.0	35.5	48.5
	2.0		RM	6.7	39.6	46.3
			Av.	9.6%	35.0%	44.6%
	2.0	Fatty Meal[d]	DS	9.4	69.4	78.8
	3.0		PG	4.9	55.3	60.2
	3.0		AM	5.1	38.8	43.9
	3.0		NM	3.5	36.5	40.0
	3.0		EH	5.6	33.2	38.8
	3.0		EHw	8.0	35.2	43.2
			Av.	6.1%	44.7%	50.8%

[a] Daily excretion values corrected by individual control figure obtained on urine and stools from day preceding drug administration.

[b] Fasting regimen indicates that the subject ate a normal meal at 6:00 p. m. and took no additional food until noon the following day. Drug was administered at 6:00 a. m. during fasting period.

[c] Standard regimen prescribed that the subject eat a light, fat-free meal at 6:00 p. m., take the drug at 10:00 p. m. and eat nothing until 8:00 a. m. the following day. Water permitted *ad libitum*.

[d] Fatty Meal regimen required that the subject take the drug midway through the 6:00 p. m. meal and that the meal be rich in fats such as butter, cream, bacon, etc.

%) and then decreased until at twenty-four to forty-eight hours no iodine could be detected by the Bang and Georg method. After this period, i.e., after forty-eight hours, PBI determinations were initiated, and the mean PBI values for the four subjects were found to be 166, 65, and 12[3] μg. % for two, twelve and ninety-eight days following drug administration, respectively. Control PBI values in these studies averaged approximately 6.5 μg. %.

Iodine blood levels following iodopanoic acid were studied on the first group of four subjects who had received the single 3.0-Gm. oral dose of this compound, and from Fig. 4, it will be seen that the maximum level was attained at approximately ten hours and decreased quite slowly so that even at seventy-two hours a measurable amount of circulating iodine was found with the Bang and Georg method. Plasma bound iodine levels were followed weekly for the first three weeks with the mean PBI for the four subjects being 160, 83, 42, and 10 μg. % for days seven, fourteen, twenty-one, and fifty-six,[4] respectively.

Urine and Fecal Elimination of Teridax® and Iodopanoic Acid in Dogs.—The excretion of Teridax® in dogs (Fig. 5) occurs slowly with only small amounts (11–14%) of the dose being recovered even up to twenty-three days after oral drug administration (150 mg./Kg.). At twenty-three days amounts of iodine were found which in some cases were greater than had been observed on the first and second days of the experiment. Fecal excretion was more pronounced in dogs than in man being approximately 1.5–4.0% of the dose, but even this amount is small when compared to the urine recoveries.

[3] This figure is the mean for three of the four subjects only because the sample for the fourth subject was lost.

[4] This figure is a mean of two subjects; the remaining subjects were not available for sampling.

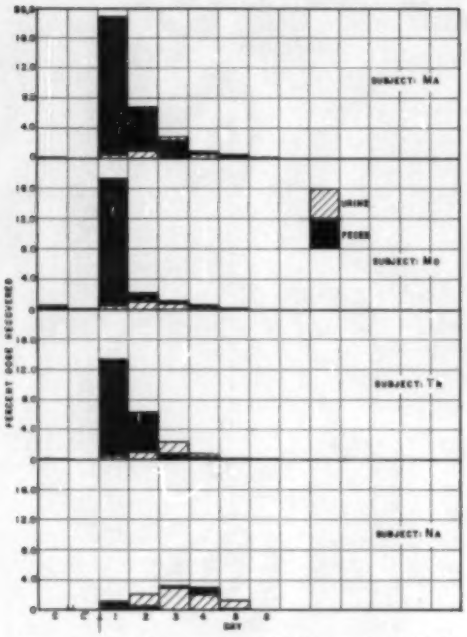

Fig. 2.—Total excretion of iodopanoic acid by four normal persons following a single oral dose of 3 Gm.

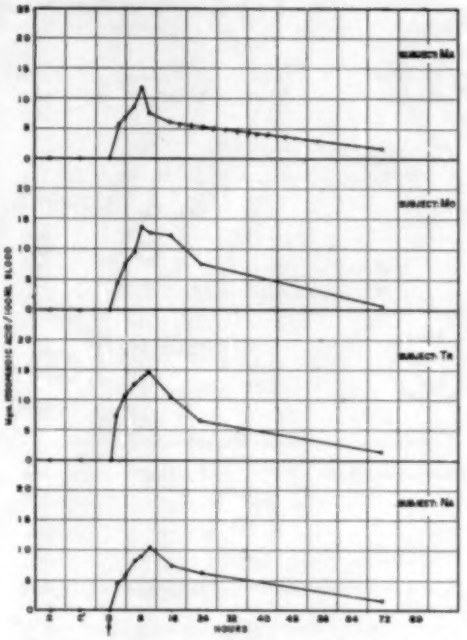

Fig. 4.—Iodopanoic acid concentration in the blood of four normal persons following oral administration of a single 3-Gm. dose.

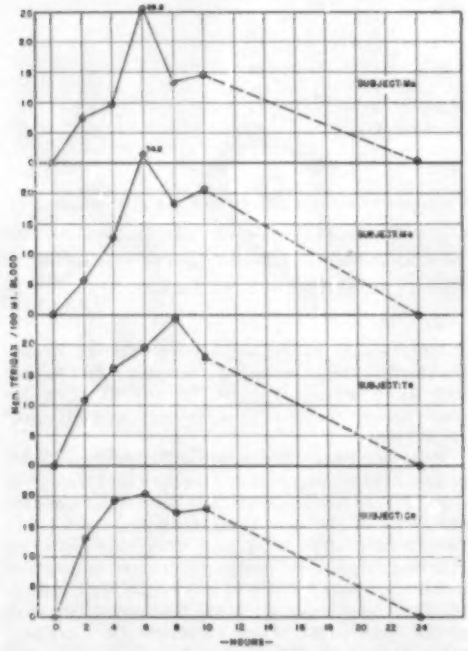

Fig. 3.—Teridax® concentration in the blood of four normal persons following oral administration of a single 3-Gm. dose.

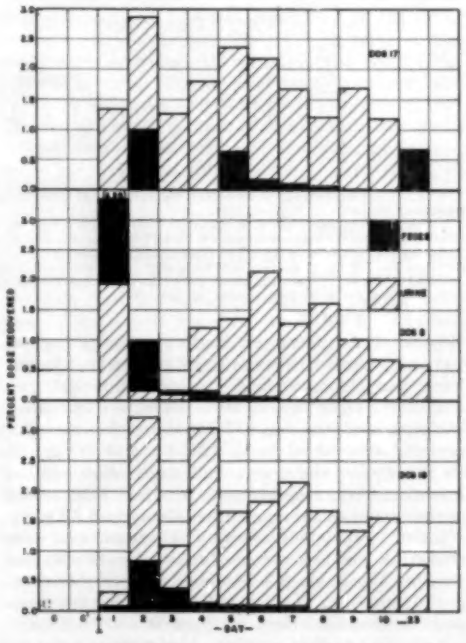

Fig. 5.—Total excretion of Teridax® by three dogs following an oral dose of 150 mg./Kg. body wt.

Iodopanoic acid, somewhat like Teridax®, when administered to dogs is slowly excreted (Fig. 6). Excretion occurs, however, unlike Teridax®, mainly in the feces. At one week following oral administra-

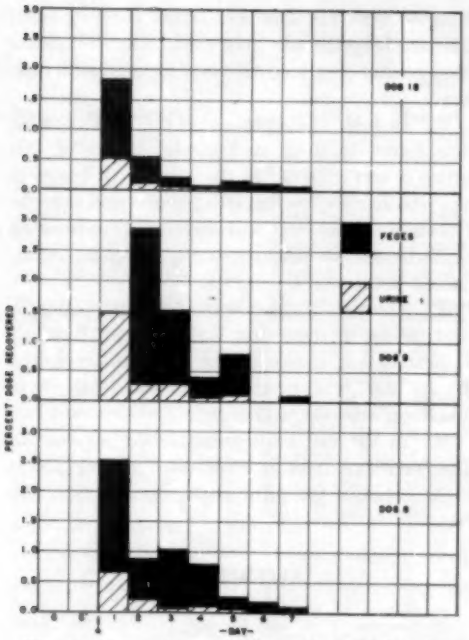

Fig. 6.—Excretion of iodopanoic acid by three dogs following an oral dose of 300 mg./Kg. body wt.

tion of the drug (300 mg./Kg.) over 90% of the drug was still unaccounted for.

Excretion of Teridax® by Rats.—Studies on rats with Teridax® were carried out because the rat does not possess a gall bladder, and it was of interest to ascertain if the excretion pattern observed in dogs and man with Teridax® might differ in the rat.

In one experiment rats were fed Teridax® (130–160 mg.) in the diet. Urine and fecal excretion of the compound were estimated, and it was found that the rats excreted 53–72% of the drug in the urine during the first forty-eight hours. In the feces only traces were noted. These results with Teridax® in rats are certainly of an entirely different order of magnitude of those found for the dog and for man with the same compound. No experiments with iodopanoic acid on rats were carried out.

It would appear that the rat is not at all satisfactory as an experimental animal in which to study excretion of this radiopaque substance. The dog, on the other hand, appears to have an excretion pattern similar to man.

Tissue Concentrations of Teridax® in Dogs.—Because only approximately 25% of the administered iodine was accounted for in the urine and stools of dogs despite long collection periods (twenty-three days), attempts to localize the remaining 75% were made. Twenty-three days after the oral administration of Teridax® (150 mg./Kg.) to four dogs and at a time when urine and fecal output of iodine was no longer evidenced in the feces and only traces were present in the urine, the animals were sacrificed and the iodine content of various tissues estimated by the Bang and Georg method (3).

Data obtained from tissue analyses of dogs 19 and 20 (Table II) did not yield a satisfactory balance although it was clearly indicated that the fat, muscle, and liver were major storage sites. It was then realized that at least one possible large tissue storage site, the skin, had not been examined; and for this reason, dogs 25 and 28 were examined in a manner similar to dogs 19 and 20 but, in addition, in dogs 25 and 28 representative skin samples and the entire hind leg of each dog were analyzed for iodine. The results of these analyses are also presented in Table II. It will be noted that on a concentration basis the iodine level in the skin and liver appear to be much greater than that found for fat and muscle. Only trace amounts of the drug appeared in the brain and heart; none could be detected in the stomach, cerebrospinal fluid, blood, small intestine, pancreas, kidney, adrenal, gall bladder, and spleen.

In an attempt to arrive at a balance on the compound, it appeared reasonable to consider the concentration of iodine in an aliquot of the ground leg as a rough approximation of the amount of compound per gram of eviscerated carcass. Then, by simple calculation, the amount of iodine (expressed as Teridax®) could be calculated. This amount, plus that found in the liver, was taken to indicate the total amount of compound in the body at the time of autopsy. Adding to this the amount eliminated in the urine and stools, an estimate of the net-balance was obtained. From the results obtained in dogs 25 and 28, it would appear that at least a rough net-balance was obtained and that the drug previously unaccounted for by urine and fecal excretion could be accounted for now in certain tissues of the body.

SUMMARY

1. Information is presented on the mode and rate of absorption, excretion, and blood levels (including plasma bound iodine) of the chole-

TABLE II.—DISTRIBUTION OF TERIDAX® IN DOGS FOLLOWING A SINGLE ORAL DOSE

Dog No.	Dose (Gm.)	Dose mg./Kg.	Teridax® (mg. %) Skin	Liver	Fat	Muscle	Leg	Teridax® (mg.) Skin[a]	Liver	Fat	Muscle	Evisc.[b] Carcass	Urine + Feces	Teridax® Balance B + E + F (Gms.)
25	2.1	300	49	80	18	11	25	280	223	255	320	1700	700	2.6
28	4.8	"	30	44	10	8	17	384	277	310	504	2600	600	3.5
19	2.6	"	...	28	7	3	...	...	77	336	287	...	390	...
20	3.3	"	...	23	6	3	...	...	106	375	320	...	396	...
									A	B	C	D	E	F

[a] Calculated on basis of skin comprising 8% body weight, fat 20% body weight, and muscle 40% body weight.
[b] Calculated from concentration in leg and assuming equivalence to eviscerated carcass in composition.

cystographic agent, Teridax®, following oral administration to man. Data on absorption and excretion are also presented for the dog and rat. In addition, certain comparative data for iodopanoic acid are given.

2. Following a single oral dose (3.0 Gm.) of Teridax® to fasting adult human males, 17–23 per cent of the administered compound, estimated as iodine, was recovered in the first forty-eight-hour urine. A total urine and stool excretion of 25 per cent was realized during a twenty-three-day collection period.

3. Blood levels of iodine following the oral ingestion of Teridax® reached a maximum at six to eight hours and decreased until at twenty-four to forty-eight hours no iodine could be found. With a more sensitive method, however, it was found that elevated plasma bound iodine levels persist for at least ninety-eight days.

4. Following a single oral dose (3.0 Gm.) of iodopanoic acid to fasting adult human males, 22–29 per cent of the dose appeared in the stools with only very small amounts appearing in the urine. Blood iodine levels reached a maximum at about ten hours and declined slowly.

5. Data on the effect of diet regime on excretion of Teridax® and iodopanoic acid in adult human subjects are presented, which indicate that greater drug retention is associated with fasting.

6. In dogs, 11–14 per cent of the Teridax® dose was found in urine and stools. Most of this amount was excreted in the urine with traces in the stools. Following iodopanoic acid administration, 10 per cent was accounted for in urine and stools; the predominant portion was eliminated in the stools.

7. Analyses of the carcasses and major organs and tissues of dogs that had received Teridax® indicated that almost all the iodine unaccounted for by stool and urine analyses was localized in the liver, skin, fat, and muscle.

8. In the rat, approximately 60 per cent of the Teridax® dose is eliminated during forty-eight hours in the urine with only traces in the feces.

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The Optical Crystallographic Properties of Some N. F. X Substances*

By JOHN A. BILES†

The optical crystallographic properties of anthralin, butyl aminobenzoate, danthron, drocarbil, hexestrol, histidine monohydrochloride, mestilbol, naphazoline hydrochloride, nitrofurazone, para-aminohippuric acid, para-nitrosulfathiazole, phthalylsulfacetamide, quinine dihydrochloride and quinine hydrochloride are reported. All compounds except mestilbol and quinone hydrochloride will be in the N. F. X. These data afford a rapid and specific method for identification.

TO AID IN THE identification of many of the U. S. P. solid substances, the optical crystallographic properties were included in the fourteenth revision (1). The use of the refractive index of solids as an aid in identification was reported in the ninth edition of the National Formulary (2). The purpose of this communication is to report the data of some substances included in the N. F. X which have not been reported in the literature.

* Received August 27, 1954, from the Pharmaceutical Chemistry Laboratories of the University of Southern California School of Pharmacy, Los Angeles, Calif.
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The author is grateful to R. N. Castle who located ample supplies of anthralin and butyl aminobenzoate from Abbott Laboratories, *p*-nitrosulfathiazole from Breon, naphazoline hydrochloride from Ciba, *p*-aminohippuric acid from Delta Chemical Works, nitrofurazone from Eaton, phthalylsulfacetamide from Schering and danthron and drocarbil from Winthrop-Stearns.

EXPERIMENTAL

The compounds studied meet N. F. specifications. The optical crystallographic properties were determined with the use of a Spencer petrographic microscope. The principal indexes of refraction were determined by the immersion method using centered interference figures to determine the proper orientation of the crystal. In some instances the index for alpha and gamma could be determined only with crystalline fragments showing high birefringence. To orient properly some of the crystalline substances, glass wool was used.

Two sets of immersion oils were used. One set was prepared using mineral oil, α-bromnaphthalene, methylene iodide, sulfur, etc. The mineral oil and α-bromnaphthalene mixtures were prepared in this laboratory. The oils of indexes 1.71 to 1.80 were purchased from Cargille Laboratories (Series M). The indexes of the purchased oils differed by an in-

TABLE I.—OPTICAL CRYSTALLOGRAPHIC DATA

Compound	System	Habit	Common Orientation	Orientation of Ellipsoid and Extinction Angle	Dispersion
Anthralin	Orthorhombic	Lamellar	Bxa	XX ‖a, YY ‖b, ZZ ‖c	Rhombic, r > v
Butyl aminobenzoate	Monoclinic	Tabular	Bxo[a]	YY ‖b, ZZ∧c, 16°	None observed
Danthron	Tetragonal	Lamellar	Optic axis	Parallel and complete	None observed
Drocarbil					
Phase I	Monoclinic	Tabular	Bxo	XX ‖b, YY∧c, 15°	Crossed, v > r
Phase II	Orthorhombic	Tabular	Bxa	XX ‖b, YY ‖c, ZZ ‖a	None observed
Hexestrol (from $CHCl_3$)	Monoclinic	Tabular	Optic axis	YY ‖b, XX∧c, 23°	None observed
Histidine monohydrochloride	Orthorhombic	Tabular	Optic axis	XX ‖a, YY ‖c, ZZ ‖b	Rhombic, r > v
Mestilbol	Monoclinic	Tabular	Optic axis	YY ‖b, XX∧c, 18°	Inclined, v > r
Naphazoline hydrochloride	Triclinic[b]	Tabular	Bxa[a]	.....	None observed
Nitrofurazone	Monoclinic	Lamellar	Optic axis	YY ‖b, ZZ∧c, 42°	None observed
para-Aminohippuric acid	Orthorhombic[c]	Columnar	Optic normal[a]	XX ‖c, YY ‖a, ZZ ‖b	Rhombic, r > v
para-Nitrosulfathiazole	Triclinic	Equant	Optic axis[a]	.....	Anomalous, very strong
Phthalylsulfacetamide	Monoclinic	Columnar	Bxa	XX ‖b, YY∧c, 8°	Horizontal, r > v
Quinine dihydrochloride	Monoclinic	Lamellar	Bxo	XX ‖b, YY ‖c, ZZ∧a, 40°	Crossed, v > r
Quinine hydrochloride	Orthorhombic	Lamellar	Bxo	XX ‖a, YY ‖b, ZZ ‖c	Rhombic, r > v

Compound	Elongation	Axial Angle	Optic Sign	Refractive Indexes[d] α	β	γ	Birefringence
Anthralin	Bxa(±), Bxo(−), O.N.(−)	Medium	+	1.580	1.690	>1.80	>0.220
Butyl aminobenzoate	Bxa(+), Bxo(−), O.N.(+)	Small	+	1.517	1.551	>1.80	>0.283
Danthron	Flash figure(−)	0°	+	1.79 (ω)	>1.80	...	>0.01
Drocarbil							
Phase I	Negative	53°	−	1.496	1.710	1.780	0.284
Phase II	Bxa(±), Bxo(−), O.N.(−)	41°	+	1.597	1.610	1.710	0.113
Hexestrol (from $CHCl_3$)	Bxa(±), Bxo(−), O.N.(−)	80°	+	1.495	1.565	1.687	0.192
Histidine monohydrochloride	Bxa(−), Bxo(+), O.N.(+)	80°	−	1.566	1.597	1.623	0.057
Mestilbol	Bxa(−), Bxo(+), O.N.(−)	52°	+	1.522	1.563	1.780	0.258
Naphazoline hydrochloride	±	Small	+	1.615	1.623	>1.780	>0.165
Nitrofurazone	±	~90°	±	1.430?	>1.80	>1.80	>0.370
Para-Aminohippuric acid	Bxa(−), Bxo(±), O.N.(−)	Medium	+	1.509	1.613	>1.80	>0.291
Para-Nitrosulfathiazole	±	Large	−	1.557	1.711	>1.80	>0.243
Phthalylsulfacetamide	Bxa(−), Bxo(+), O.N.(−)	≤25°	+	1.550	1.568	≧1.80	≧0.250
Quinine dihydrochloride	Bxa(±), Bxo(−), O.N.(−)	52°	−	1.542	1.657	1.689	0.147
Quinine hydrochloride	Positive	61°	+	1.590	1.610	1.669	0.079

[a] Off centered. [b] Pseudomonoclinic. [c] Orthorhombic sphenoidal. [d] Indexes determined at 5889, 5895 Å. (sodium vapor light).

terval of 0.1. The second set of oils was prepared by mixing varied amounts of glycerin and a solution of potassium mercuric iodide. The choice of the oils depended on the solubility of the solid substance.

The indexes of refraction were determined approximately using white light, then determined accurately using sodium vapor light of wavelength 5,889 and 5,895 Å. The indexes were determined at or corrected to 25° ± 1°. The accuracy of the determinations was ±0.002, excepting the determinations of the indexes between 1.71 and 1.80. The accuracy for these high values was ±0.005 because of the 0.1 intervals of the oils. Utilizing the indexes the axial angle, 2V, was calculated using the more exact formula suggested by Wahlstrom (3). The birefringence, which is the numerical difference between the highest and lowest indexes, is reported because the value together with the optic sign may be valuable in predicting the orientation of the molecule in the solid state. This is particularly true with aromatic compounds.

The method of reporting elongation refers to the interference figure observed. The habit is described following the suggestion by Winchell (4). No pleochroism was observed in any of the compounds except *p*-nitrosulfathiazole which possibly may be dichroic.

Table I lists the most important optical crystallographic data of the compounds.

DISCUSSION

The optic sign of nitrofurazone could not be determined. The birefringence is very large. This is expected because of the partial planarity and conjugation. It was very difficult to determine alpha because the lamellar crystals must be oriented at an angle of 42°. In addition the oils of low index of refraction are quite volatile.

The properties of most of the sulfonamide drugs have been reported (5). It is interesting to note that alpha and beta for phthalylsulfacetamide and sulfacetamide are almost identical. A nitrogen assay eliminated the possibility of substitution or hydrolysis. The crystal system for *p*-nitrosulfathiazole was determined from the dispersion. This substance shows very strong anomalous dispersion, such dispersion shown by triclinic crystals. The orientation of the bisectrix in *p*-nitrosulfathiazole could not be determined. For exact determination of the system and orientation of the bisectrix, one must resort to goniometric measurements or X-ray studies.

Naphazoline hydrochloride is pseudomonoclinic. Measurement of the angles indicated that the crystal was not monoclinic. Naphazoline hydrochloride reacted with the immersion oils of indexes greater than 1.78. The indexes, system, sign, habit, and elongation for quinine monohydrochloride have been reported (6). The additional data for this U. S. P. substance are herewith reported. The quinine dihydrochloride was recrystallized from a mixture of alcohol and chloroform. To a saturated solution was added enough ether to cause precipitation. Excellent crystals were obtained following twenty-four hours' standing.

The properties of the synthetic hormones are valuable in discussion of molecular orientation. The optical crystallographic data for estrone have been reported (7). The three reported phases of estrone have a negative optic sign. The crystal properties of diethylstilbestrol are similar to those of estrone (8). Both hexestrol and monomestrol are positive crystals.

Two definite crystalline phases were observed with drocarbil. The two phases occurred from the recrystallization from alcohol. A separation of phases was not successful. Therefore, analyses could not be made.

SUMMARY

1. The optical crystallographic data are reported for thirteen N. F. X solid substances and one U. S. P. XV solid substance.

2. The data afford a rapid and specific method of identification.

3. The data can be determined on small amounts of material.

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Mercurated Amides of Alpha-Amino Acids*

By WILLIAM O. FOYE and ROBERT A. MODE†

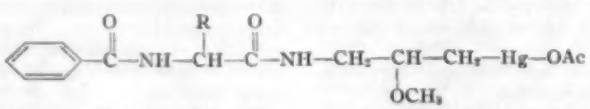
Naturally-occurring α-amino acids have been employed in the preparation of a series of mercurated allyl amides as possible diuretics with low toxic and irritant properties. Relatively stable, recrystallizable and water-soluble mercurials were isolated which showed diuretic activity either by intravenous or oral administration.

THE ORGANIC MERCURIALS have been accepted as one of the most effective types of agents for the stimulation of diuresis in the relief of edema (1), but occasional toxic reactions (2) often cardiovascular in nature, limit their usefulness. The report of Wachstein and Meisel (3) that the presence of certain α-amino acids alleviated the toxicity of mercurial diuretics in rats made promising a study of mercurated derivatives of α-amino acids themselves. Furthermore, the studies of Rowland (4) which indicated that the toxicity of mercurial diuretics was not due directly to the ionization of the mercury also suggested the use of relatively nontoxic organic components for the preparation of mercurial diuretics. A series of mercurated derivatives of α-amino acids having the following structure (I) has accordingly been prepared. These compounds are named as N-(3-acetoxymercuri-2-methoxypropyl)-alpha-substituted hippuramides in this communication.

The majority of organic mercurials which have seen clinical use are 3-substituted mercuri 2-methoxypropylamides of organic acids (which are designated as mercurated allyl amides in this discussion), since the allyl group provides a convenient seat of attachment for the mercury. Although a variety of organic acids have been employed in possible diuretics of this type, no α-amino acids have been reported for this purpose. Some closely related types which have shown good diuretic activity, however, are mercurated N-allyl urethans (5) and mercurated derivatives of N-allyl urea (6).

DISCUSSION

Attempts to prepare mercurated allyl amides of α-amino acids in which the amino group is unprotected produced only noncrystallizable, nonpurifiable oils. When the amino groups were blocked with the benzoyl group, however, the desired products were readily obtained by amide formation with



(I)

allyl isothiocyanate and mercuration with mercuric acetate in methanol. The exact structure of methoxy-mercurated allyl amides has been proved by Pearson, *et al.* (7).

The major problem encountered in this synthesis was the isolation of the mercurated product in crystalline form. The oils which resulted after re-

* Received August 27, 1954, from the School of Pharmacy, University of Wisconsin, Madison.
Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.
Abstracted from the thesis presented to the Graduate School of the University of Wisconsin by Robert A. Mode in partial fulfillment of the requirements for the degree of Master of Science, January, 1953.
† Eli Lilly & Co. Fellow, 1951–1952; Fellow of the American Foundation for Pharmaceutical Education, 1952–1953.

TABLE I.—N-(3-ACETOXYMERCURI-2-METHOXYPROPYL)-HIPPURAMIDES (I)

Amino Acid Used	Yield, %[a]	m. p.[b]	Recrystn. Solvent	Formula	Analyses, % Calcd.	Found[c]
Glycine	30	139–142°	Methanol-ether	$C_{15}H_{20}O_6N_2Hg$	C: 35.41	35.63
					H: 3.93	4.12
Alanine	48	146–148°		$C_{16}H_{22}O_6N_2Hg$	C: 36.73	36.49
					H: 4.24	4.14
dl-Leucine	88	154–156°	Methanol-ether	$C_{19}H_{28}O_6N_2Hg$	C: 40.38	40.48
					H: 5.00	5.26
dl-Methionine	58	123–127°		$C_{16}H_{26}O_6N_2SHg$	C: 37.06	37.64
					H: 4.50	4.20
dl-Phenylalanine	77	164–166°	Acetone	$C_{22}H_{24}O_6N_2Hg$	C: 44.11	44.41
					H: 4.37	4.55

[a] Calculated from the allyl amides of the α-benzoylamino acids.
[b] All melting points are corrected.
[c] Analyses for C and H were carried out by the Clark Microanalytical Laboratory, Urbana, Ill.

fluxing the methanol solutions of the allyl amides and mercuric acetate were eventually found to crystallize from ether-methanol solutions of the correct proportion to give faint turbidity, after prolonged scratching and cooling. When the mercurials were obtained without refluxing but by prolonged standing at room temperature, lower melting products resulted which gave the same analysis after thorough drying. These products are believed to be hydrates. In either case, the mercurated amides were relatively stable, recrystallizable, and water-soluble. The physical characteristics and analytical proof of the compounds prepared are listed in Table I.

The diuretic activity of these compounds, determined at the Lilly Research Laboratories by Drs. K. K. Chen and E. B. Robbins, was in general described as good, although the leucine and phenylalanine derivatives had a weak effect. The results of intravenous administration in dogs is summarized in Table II. The glycine derivative, the most promising of these diuretics, also produced diuresis in oral doses of 2 mg./Kg. in one of four dogs without signs of gastric distress. Oral doses of 4 mg./Kg. caused diuresis in three of four dogs and gastric distress in two of four. No gastric distress was observed from the intravenous doses. The acute oral toxicity of the glycine derivative in 1% solution was approximately 350 mg./Kg. in the mouse.

TABLE II.—DIURETIC ACTIVITY OF THE MERCURATED AMIDES

Mercurated Amide	Dose,[a] mg./Kg.	Diuresis Obs./No. of Dogs	Dose,[a] mg./Kg.	Diuresis Obs./No. of Dogs
Glycine	0.5	2/6	1.0	6/6
Alanine	0.5	3/6	1.0	4/6
Methionine	0.5	3/6	1.0	3/6
Leucine	0.5	1/2	1.0	1/2
Phenylalanine	0.5	0/2	1.0	2/2

[a] The compounds were dissolved in dimethylformamide and administered intravenously.

EXPERIMENTAL

α-Benzoylamino Acids.—Benzoylation of the *dl* α-amino acids was carried out by the general procedure of Ingersoll and Babcock (8) for hippuric acid, except that the reaction mixtures were shaken, instead of being stirred, until the odor of benzoyl chloride had disappeared. The following yields and melting points were obtained after recrystallization from aqueous alcohol: N-benzoyl alanine, 89%, 165–167°; N-benzoyl leucine, 86%, 141–143°; N-benzoyl methionine, 79%, 152.5–154°; N-benzoyl phenylalanine, 92%, 186–187.5°. Several of these melting points are higher than those previously reported.

N-Allyl Hippuramides.—The general procedure of Diels and Beccard (9) was used. In a 200-cc. flask equipped with a reflux condenser and a gas collecting attachment were placed 50 Gm. (0.28 mole) of hippuric acid and 30 Gm. (0.30 mole) of freshly distilled allyl isothiocyanate (b. p. 150.7°). The mixture was heated at 130° until no more gas, carbon oxysulfide, was evolved (generally two hours were required). After cooling to room temperature, the impure product was dissolved in boiling acetone and precipitated by petroleum ether. After several such precipitations and recrystallization from aqueous alcohol, 36.8 Gm. (60%) of white, crystalline N-allylhippuramide was obtained; m. p. 133–135° (Lit., m. p. 138.5°).

N-allyl-α-methylhippuramide, after recrystallization from acetone, was obtained in 50% yield as white, mossy needles; m. p. 139–140.5°.

Anal.—Calcd. for $C_{13}H_{16}O_3N_2$: C, 67.22; H, 6.94. Found: C, 67.33; H, 7.04.

N-Allyl-α-isobutylhippuramide, recrystallized from acetone, was obtained in 60% yield as glistening, white crystals; m. p. 154–155°.

Anal.—Calcd. for $C_{16}H_{22}O_3N_2$: C, 70.04; H, 8.08. Found: C, 70.07; H, 8.14.

N - Allyl - α - (β′ - methylmercaptoethyl) - hippuramide, recrystallized from acetone, was obtained in 58% yield as white needles; m. p. 136.5–138°.

Anal.—Calcd. for $C_{13}H_{20}O_3N_2S$: C, 61.61; H, 6.89. Found: C, 61.38; H, 7.07.

N-Allyl-α-benzylhippuramide, white needles, was obtained in 62% yield after recrystallization from acetone; m. p. 164–166°.

Anal.—Calcd. for $C_{19}H_{20}O_3N_2$: C, 74.00; H, 6.54. Found: C, 73.98; H, 6.14.

Mercuration of the Allyl Amides.—The preparation of the glycine derivative is representative of this procedure. To a hot solution of 10 Gm. (0.046 mole) of N-allylhippuramide in 200 ml. of absolute methanol was added a hot solution of 14.5 Gm. (0.045 mole) of mercuric acetate in 200 ml. of absolute methanol. The mixture was warmed gently for ten minutes and then allowed to cool to room temperature, when a small quantity of flaky precipitate appeared. After filtration, the solution

was allowed to stand overnight. It was then refluxed for two to three hours, when another small amount of gray precipitate formed and was removed. The resulting solution was concentrated at 30° (40 mm. pressure) to a viscous oil which was dried at room temperature under vacuum (1 mm.) to remove any remaining acetic acid. The product was dissolved in hot methanol, and anhydrous ether was added to the point of lasting turbidity. After prolonged cooling (generally over two days) and scratching, white crystals (7.3 Gm., 30%) were obtained which could be recrystallized from methanol-ether solution. The melting point was 139–142°. When the refluxing period was shortened or omitted and the oil was not dried, a melting point of 116–122° was obtained.

SUMMARY

1. A series of mercurated allyl amides of α-amino acids has been prepared for evaluation of diuretic activity.

2. The following new compounds have been characterized: N-(3-acetoxy-mercuri-2-methoxypropyl)-hippuramide, N-allyl-α-methylhippuramide, N-(3-acetoxymercuri-2-methoxypropyl)-α-methylhippuramide, N-allyl-α-isobutylhippuramide, N-(3-acetoxymercuri-2-methoxypropyl)-α-isobutyl-hippuramide, N-allyl-α-(β′-methylmercaptoethyl) - hippuramide, N-(3-acetoxymercuri-2-methoxypropyl)-α-(β′-methylmercaptoethyl) - hippuramide, N - allyl - α - benzylhippuramide, and N-(3-acetoxymercuri-2-methoxypropyl)-α-benzylhippuramide.

3. These compounds showed good diuretic activity and low toxic and irritant properties by intravenous administration in dogs. The mercurated glycine amide also showed good diuretic activity by oral administration.

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The Synthesis of Some New Alkyl *p*-N-Alkyl Amidinobenzoates*

By ROBERT BOBLITT† and OLE GISVOLD‡

The following compounds have been prepared and reported: the *n*-amyl, iso-amyl, *n*-hexyl, *n*-heptyl, *n*-octyl, and cyclohexyl derivatives of the benzyl, hexahydrobenzyl, and cyclohexyl esters of *p*-carboxybenzamidine. If possible, these compounds will be screened for anesthetic and antibiotic properties and the results reported later.

PREVIOUS publications (1, 2) have described the synthesis of some esters of some N-alkyl *p*-carboxybenzamidines. Prolonged surface and infiltration anesthesia, approximately four hundred minutes, was obtained by certain esters when the alcohol portion was benzyl, hexahydrobenzyl, or cyclohexyl.

Following an initial drop in activity by N-methylation or N-ethylation, there is a uniform increase in the duration of activity with an increase in the size of the N-alkyl substituent. The largest alkyl group that was introduced was an *n*-butyl group. These N-alkyl derivatives exhibited maximum activity. It was therefore of interest to determine the size and nature of the N-alkyl groups that would lead to maximum surface and intracutaneous activities.

* Received June 4, 1954, from the University of Minnesota, College of Pharmacy, Minnesota.
Abstracted from a dissertation submitted to the Graduate Faculty of the University of Minnesota by Robert Boblitt in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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EXPERIMENTAL

The preparation of the intermediates such as *p*-cyanobenzoic acid, *p*-cyanobenzoyl chloride, alkyl-*p*-cyanobenzoates, alkyl-*p*-carbethoxyiminobenzoates and amidines were prepared essentially according to previously described procedures. However, some improvements and variations are reported as follows.

Alkyl-*p*-cyanobenzoates.—Improved yields, almost quantitative, of these intermediates were obtained when two equivalents of the alcohol and one equivalent of *p*-cyanobenzoyl chloride were refluxed in benzene.

Alkyl-*p*-carbethoxyiminobenzoates.—It should be recorded here that the use of anhydrous ether, as in Pinner's original method, gave considerably greater yields of the imidic esters than when anhy-

TABLE I.—ALKYL *p*-ALKYLAMIDINOBENZOATES

No.	Alkyl Residue	Yield, %	m. p., °C.	Analysis, % Calcd.	Found
Benzyl Ester of *p*-N-Alkyl Amidinobenzoic Acid Hydrochlorides HCl·HNR—C(=NH)$C_6H_4COOCH_2C_6H_5$					
1	*n*-Amyl	79.0	199–200	C, 66.56 H, 6.98	C, 66.56 H, 7.21
2	Isoamyl	41.0	210	C, 66.56 H, 6.98	C, 66.50 H, 7.15
3	*n*-Hexyl	69.5	204	C, 67.26 H, 7.26	C, 67.10 H, 7.47
4	*n*-Heptyl	62	208–209	C, 67.94 H, 7.52	C, 67.75 H, 7.56
5	*n*-Octyl	80	207	C, 68.55 H, 7.75	C, 68.64 H, 8.01
6	Cyclohexyl	53.5	243–244	C, 67.64 H, 6.76	C, 67.70 H, 6.80
Hexahydrobenzyl Ester of *p*-N-Alkyl Amidinobenzoic Acid Hydrochlorides HCl·HNR—C(=NH)$C_6H_4COOCH_2C_6H_{11}$					
1	*n*-Amyl	98	245	C, 65.46 H, 8.52	C, 65.52 H, 8.55
2	Isoamyl	95	259	C, 65.46 H, 8.52	C, 65.31 H, 8.72
3	*n*-Hexyl	92	222	C, 66.23 H, 8.73	C, 66.08 H, 8.70
4	*n*-Heptyl	86	218	C, 66.90 H, 8.93	C, 66.66 H, 8.91
5	*n*-Octyl	79	211	C, 67.54 H, 9.12	C, 66.86 H, 9.17
6	Cyclohexyl	81	292–293	C, 66.56 H, 8.25	C, 66.48 H, 8.24
Cyclohexyl Ester of *p*-N-Alkyl Amidinobenzoic Acid Hydrochlorides HCl·HNR—C(=NH)$C_6H_4COOC_6H_{11}$					
1	*n*-Amyl	80	239–240	C, 64.66 H, 8.28	C, 64.50 H, 8.30
2	Isoamyl	70	253–254	C, 64.66 H, 8.28	C, 64.84 H, 8.36
3	*n*-Hexyl	84.5	242–243	C, 65.46 H, 8.52	C, 65.30 H, 8.73
4	*n*-Heptyl	42	236–237	C, 66.21 H, 8.75	C, 66.49 H, 8.75
5	*n*-Octyl	73.5	230–231	C, 66.90 H, 8.93	C, 66.94 H, 8.95
6	Cyclohexyl	52.0	288–289	C, 65.82 H, 8.02	C, 66.05 H, 8.09

drous dioxane was used. Furthermore, the ease of manipulation was greater with ether than with dioxane. When ether was used as a solvent, excellent yields of the imidic ester hydrochlorides were obtained after the reaction mixture had stood in the refrigerator for three days.

Alkyl-*p*-N-alkyl Amidinobenzoates.—Some of these compounds were first prepared by the procedure published by Rost and Gisvold (1). However, the following modification gave higher yields of the desired amidines. One equivalent of the amine was added to the ethanol before the imidic ester hydrochloride was dissolved. Additional amine was then added dropwise until a *p*H of 7.0–7.5 was obtained. After the usual heating period of four hours at 70°, the solvent was removed and ether was added. In the case of the *n*-amyl, isoamyl, and cyclohexyl N-alkyl derivatives, crystalline products were obtained upon refrigeration for several hours. In the case of the *n*-hexyl, *n*-heptyl, and *n*-octyl N-alkyl derivatives, refrigeration did not yield crystalline products. The addition of petroleum ether followed by a second period of refrigeration yielded crystalline products. The above crystalline products, amidine hydrochlorides, were dissolved in a minimum amount of hot water. Upon cooling, these salts readily separated as crystalline materials.

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Anticonvulsants*

A Study of Some Alpha, Alpha-Disubstituted Malondiamides

By HERBERT. F. SCHWARTZ† and ROBERT F. DOERGE‡

The primary purpose of this study was the preparation of a series of α,α-disubstituted malondiamides not previously prepared nor investigated for their pharmacological properties. Because of their structural relationships to well-known compounds having anticonvulsant activity, and furthermore, since two of the lower members of the series have been shown to be without hypnotic activity, a characteristic desirable in a good anticonvulsant, this series was considered worthy for further investigation as anticonvulsants. The prepared compounds were tested for anticonvulsant activity on the male albino rat against electroshock and against Metrazol®. Some of the compounds exhibited anticonvulsant activity. At the maximum tolerated oral dose the compounds had no action against the stimulating effect of 30 mg./Kg. of ephedrine sulfate injected subcutaneously.

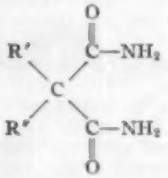
IN THE MAIN, the proper use of anticonvulsant drugs is the prime consideration in the management of epilepsy. The first effective medicinal agents to be used were the inorganic bromides, which were introduced in England about 1857. The introduction of phenobarbital in 1912 soon led to the replacement of the bromides; in fact, phenobarbital is still considered to be a very valuable drug, while the bromides are rarely, if ever, used in the control of epilepsy. Research in the field of anticonvulsants led to the testing of certain hydantoins by Putnam and Merritt (1). From their work came Dilantin® which still remains as one of the best agents for *grand mal* epilepsy. Another hydantoin which has gained some popularity is Mesantoin® (3-methyl-5,5-phenylethylhydantoin).

Drugs which are more specific for the control of *petit mal* epilepsy also have been introduced. Tridione® (3,5,5-trimethyloxazolidine-2,4-dione) was introduced in 1945 (2), and Paradione® (3-methyl-5,5-methylethyloxazolidine-2,4-dione) in 1947 (3). More recently Milontin® (N-methyl-α-phenylsuccinimide) has been clinically evaluated (4) and is now commercially available.

Other drugs which are now commercially available include: Mebaral® (N-methyl-5,5-phenylethylbarbituric acid), Gemonil (N-methyl-5,5-diethylbarbituric acid), Mysoline® (5,5-phenylethylhexahydropyrimidine-4,6-dione), Phenurone® (phenylacetylurea), Hibicon® (N-benzyl-β-chloropropionamidex). Clinical investigation of Themisone® (α-methylmandelamide) has been reported (5).

A study of the literature shows that many of the drugs in present-day use have an undesirable sedative action, while there are others which possess little or no sedative action but do cause other undesirable side effects, such as skin rashes, photophobia, exaggeration of personality defects, liver damage, and agranulocytosis (6–11). This would indicate that there remains a need for better anticonvulsants and that further investigation of possible anticonvulsants should be carried on.

Many of the synthetic organic compounds previously mentioned as anticonvulsants possess the group —CO—NR— in common. However, this structural unit is not essential for anticonvulsant activity. Evidence of this is shown by Putnam and Merritt (1), who found activity in certain aldehydes and ketones, and also by Berger (12), working with 2,2-substituted-1,3-propanediols. With the exception of Hibicon® and Phenurone®, all of the previously mentioned anticonvulsants have a tertiary or quaternary carbon atom. The structural relationships of the α-, α-dialkylmalondiamides to the effective anticonvulsants, and the fact that the diethyl-, dipropyl- (13), and ethyl-(1-methyl)butylmalondiamide (14) have no hypnotic activity, indicated that this series was worthy of further investigation as anticonvulsants. Accordingly, a series of substituted malondiamides of the following structure was prepared in order to test their pharmacological activity:



Alpha-ethylcyanoacetamide was prepared by

* Received August 5, 1954, from the College of Pharmacy, University of Texas, Austin.
Abstracted from a thesis presented to the Faculty of the Graduate School of the University of Texas by Herbert F. Schwartz in partial fulfillment for the degree of Master of Science in Pharmacy.
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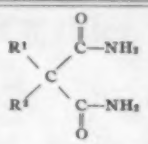
TABLE I.—PHARMACOLOGICAL DATA ON MALONDIAMIDES STUDIES[a]



Compound Number	R^1	R^2	Maximum Tolerated Oral Dose, mg./Kg.	Test Results SED[b]	SMES[c]	RSCM[d]
2661 L	C_2H_5	C_2H_5	>1000	0[e]	+	+
2657 L	C_2H_5	n-C_3H_7	200	0	+	2+
2653 L	C_2H_5	n-C_4H_9	200	0	2+	3+
2662 L	C_2H_5	i-C_4H_9	400	0	0	0
2656 L	C_2H_5	n-C_5H_{11}	250	0	4+	0
2664 L	C_2H_5	i-C_5H_{11}	>1000	0	0	0

[a] The male albino rat, dosed orally, was used in all the tests.
[b] SED–Sedative: The ability of the compound to control the stimulating action of 30 mg./Kg. ephedrine sulfate injected subcutaneously is recorded on a kymographic record.
[c] SMES–Supramaximal Electroshock Test: The ability of the compound to abolish the hind limb extensor reflex produced by a current of 150 ma delivered for 0.3 second to the brain via the optic pathway is recorded.
[d] RSCM–Rat Subcutaneous Metrazol® Test: The ability of the compound to block completely the convulsions produced by Metrazol® injected subcutaneously at a level of 70 mg./Kg. is noted.
[e] Rating System: In all the tests, 0 to + indicates essentially no detectable activity of the compound to produce the desired effect. A rating of 2+ to 3+ indicates fair to good activity. A 4+ rating indicates complete control or desired effect and from an activity standpoint is the maximum attainable rating.

condensing acetaldehyde, under reducing conditions, with cyanoacetamide. The process used is a modification of one published by Alexander and Cope (15) for the preparation of ethyl-α-ethylcyanoacetate. The optimum ratio of reactants was found to be one mole of cyanoacetamide to two moles of acetaldehyde. The dialkylcyanoacetamides were prepared from this compound by known procedures. The corresponding dialkylmalondiamides were prepared from the dialkylcyanoacetamides by treatment with cold concentrated sulfuric acid.

The malondiamides were screened for anticonvulsant activity by Dr. Raymond W. Cunningham of Lederle Laboratories, to whom the authors are indebted for the pharmacological data reported here (Table I). None of the compounds had a sedative effect when the maximum tolerated oral dose was administered. When both alkyl groups were ethyl the resulting compound was inactive to weakly active. Changing one group to normal propyl abolished any anti-Metrazol® activity. The introduction of a normal butyl group in place of an ethyl group gave a compound that possessed fair activity against both Metrazol® and electroshock; on the other hand, an isobutyl group gave a completely inactive compound. Increasing one alkyl group to normal amyl resulted in a derivative with complete control of electroshock, with no activity against Metrazol®. Again, changing the normal alkyl group to a branched chain led to a completely inactive compound. Goodman, *et al.*, (16) in a study of the anticonvulsant properties of Mysoline® reported that phenylethylmalondiamide was isolated from the urine of the test animals. The possibility that this was the active antiepileptic substance, to which Mysoline® is converted, was investigated. They concluded that such was unlikely because they found phenylethylmalondiamide to be less potent and more toxic than Mysoline®, itself.

EXPERIMENTAL[1]

Cyanoacetamide.—This compound was prepared from ethylcyanoacetate (Matheson) and concentrated ammonia water in the manner set forth in "Organic Syntheses" (17).

Ethylcyanoacetamide.—Cyanoacetamide (37.8 Gm.) was dissolved in water (285 cc.) and chilled to about 4° in a 500-cc. Pyrex bottle to be used later for the hydrogenation. Ten per cent palladium on charcoal catalyst (0.9 Gm.) (The American Platinum Works) was added and mixed by shaking, followed by previously chilled fresh acetaldehyde (40.5 Gm.) (Eastman). Without delay, the piperidine solution (1.5 cc. of 10% piperidine in glacial acetic acid) was added and hydrogenation begun at once at a pressure of one to two atmospheres, using a Parr low pressure hydrogenation apparatus.

When the reaction was complete (eight to twelve hours) the catalyst was removed by filtration. If crystals were present prior to filtration the mixture was warmed slightly. The filtrate was chilled in an ice bath or refrigerator to yield flake-like crystals. The yield was 36 to 46% with a melting point of 111–112°. Further amounts of impure ethylcyanoacetamide could be obtained by concentrating the mother liquor and chilling.

When recrystallized from hot water it melted at 113–114°. Literature reports give 112–113° (18) and 113° (19). A mixture of ethylcyanoacetamide prepared by the standard alkylation process and this compound melted at 112.5–113°.

Table II includes the disubstituted cyanoacetamides all of which were prepared from ethylcyanoacetamide using a method similar, except for the order of the introduction of the alkyl substituents, to that reported by Doerge and Wilson (18).

[1] All melting points are uncorrected.

TABLE II.—ANALYTICAL DATA ON COMPOUNDS STUDIED[a]

Compound	Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Yield %	M. p., °C.
$R(C_2H_5)C(CN)C(O)NH_2$							
R = C_2H_5			not analyzed			63.0	121.5–122[c]
R = *n*-C_3H_7	$C_8H_{14}ON_2$	62.31	62.67	9.15	9.19	88.2	113.5–114.5[d]
R = *i*-C_3H_7	$C_8H_{14}ON_2$		not analyzed			62.3	111–112[e]
R = *n*-C_4H_9[b]	$C_9H_{16}ON_2$	64.26	64.57	9.59	9.57	68.4	105–105.5
R = *i*-C_4H_9	$C_9H_{16}ON_2$	64.26	64.29	9.59	9.41	46.3	103.5–104.5[f]
R = *n*-C_5H_{11}[b]	$C_{10}H_{18}ON_2$	65.88	66.07	9.96	9.84	44.0	77–77.5
R = *i*-C_5H_{11}	$C_{10}H_{18}ON_2$		not analyzed			77.9[g]	100–101[h]
R = $CH_3CH_2CH_2CH(CH_3)$[b]	$C_{10}H_{18}ON_2$	65.88	66.48	9.96	9.98	6.6	113.5–114.5
$R(C_2H_5)C[C(O)NH_2]C(O)NH_2$							
R = C_2H_5	$C_7H_{14}O_2N_2$	53.14	53.05	8.92	9.03	quant.	218–219[i,j]
R = *n*-C_3H_7[b]	$C_8H_{16}O_2N_2$	55.80	55.77	9.37	9.23	quant.	195–196
R = *n*-C_4H_9[b]	$C_9H_{18}O_2N_2$	58.02	58.18	9.74	9.65	77.2	182–183
R = *i*-C_4H_9[b]	$C_9H_{18}O_2N_2$	58.02	58.05	9.74	9.48	61.5	211–212
R = *n*-C_5H_{11}[b]	$C_{10}H_{20}O_2N_2$	59.95	60.01	10.06	9.84	91.0	176–177
R = *i*-C_5H_{11}[b]	$C_{10}H_{20}O_2N_2$	59.95	60.38	10.06	10.01	84.0	191–192

[a] Carbon and hydrogen analyses by Micro-Tech Laboratories, Skokie, Ill. [b] Submitted as new compound. [c] Doerge and Wilson (18) report 120–121°. [d] Conrad and Zart (20) report 116°. [e] Doerge and Wilson (18) report 111–112.5°. [f] Doerge and Wilson (18) report 127.5–128.5°. [g] Initial crude yield. [h] Pure compound, Doerge and Wilson (18) report 88–89°. [i] Conrad and Zart (20) report 220°. [j] Fischer and Dilthey (21) report 224° (Corr.)

Ethylisobutylcyanoacetamide.—This compound melted at 103.5–104.5°.

Anal.—Calcd. for $C_9H_{16}N_2O$: C, 64.26; H, 9.59. Found: C, 64.29; H, 9.41. Doerge and Wilson (18) reported a melting point of 127.5° and an analysis of C, 67.36; H, 10.22.

General Method for Conversion of Cyanoacetamides to Malondiamides.—Sufficient cold, concentrated sulfuric acid was added to the corresponding cyanoacetamide in a beaker to form a clear solution. The mixture was then set aside at room temperature for several days, after which time it was poured with stirring over chipped ice. The crystalline material was collected, washed with cold, distilled water, and recrystallized from hot water.

Attempted Synthesis of Ethylisopropylmalondiamide.—The starting material was recovered unchanged. A repetition of the experiment gave similar results. No further attempts were made to prepare this compound.

Attempted Synthesis of Ethyl-(1-methylbutyl)-malondiamide.—The starting material was recovered when the reaction was run at room temperature, at 40°, and at 80°. No further attempts were made to synthesize this compound.

Table II presents the analytical data on the compounds reported.

Both chemical and pharmacological investigation is being continued on these structures.

SUMMARY

1. A procedure for the synthesis of monoethylcyanoacetamide, by the condensation of acetaldehyde with cyanoacetamide under reducing conditions, has been presented.

2. Three new dialkylcyanoacetamides were prepared.

3. Five new dialkylmalondiamides were prepared.

4. The attempted synthesis of ethylisopropylmalondiamide and ethyl-(1-methylbutyl)malondiamide from the corresponding cyanoacetamides are reported.

5. Some of the malondiamides showed anticonvulsant activity when tested on male albino rats.

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Comparison and Correlation of the Strong Cobb and the Monsanto Tablet Hardness Testers*

By ARCHIE McCALLUM, JOHN BUCHTER, and ROBERT ALBRECHT†

When testing tablets of uniform hardness and dimensions, a definite relationship was found to exist between the Strong Cobb and the Monsanto tablet hardness testers. Single instruments of either type appear to give reproducible results and multiple instruments of both types were found to check each other closely.

THE HARDNESS OF TABLETS is determined in the pharmaceutical industry usually by the Strong Cobb (1) or the Monsanto (2) testers. Both instruments give readings in terms of kilograms of pressure: the Monsanto tester measures the pressure required to compress a spring pressing the tablets and the Strong Cobb the pressure exerted directly on the tablets by a pneumatic plunger. Although both instruments give indications of tablet hardness, the values differ. This study was undertaken to compare and correlate the readings of the two instruments. To compare validly hardness determinations on both testers, the following test conditions must be met: (*a*) The sizes and weights of tablets must not vary significantly. (*b*) A single operator must be used to avoid individual variations in technique. (*c*) The tablets used for comparison purposes must be of the type which do not vary appreciably in hardness when sizes and weights are held constant. Condition (*a*) was met by segregating tablets into groups of uniform sizes and weights, while (*b*) was fulfilled by using one operator to obtain all the experimental hardness data reported in this study. Tablets of various types were studied to determine adherence to condition (*c*).

Aspirin tablets of uniform size and weight were found to vary significantly in hardness from tablet to tablet and hence could not be utilized as a standard. This was also found to be true of aspirin tablets manufactured by two other concerns. Likewise, aspirin compound tablets exhibited hardness variation from tablet to tablet.

TABLE I.—TABLET HARDNESS MEASURED WITH STRONG COBB AND MONSANTO TESTERS

Sodium Bicarbonate, 10 gr.; Standard Cup, 13/32-in. Die; Thickness, 0.211 in. Weight, 700 mg.		Ferrous Sulfate, Vitamins; Standard Cup, 13/32-in. Die; Thickness, 0.226 in. Weight, 674–678 mg.		Weight, 680–688 mg.		Sodium Salicylate, Sodium Paba; Semi-deep Cup, 1/2-in. Die; Thickness, 0.221 in. Weight, 628–634 mg.		Weight, 640–648 mg.	
Strong Cobb No. 214	Monsanto No. 2	Strong Cobb No. 214	Monsanto No. 2	Strong Cobb No. 216	Monsanto No. 1	Strong Cobb No. 216	Monsanto No. 2	Strong Cobb No. 217	Monsanto No. 1
18	10	10—3/4	6—1/2	11—1/2	8	12—3/4	6—3/4	15	9
17	11	11—1/4	7—1/2	11—1/4	7	11	6—1/2	11	6—3/4
17—1/4	10	11	7	12	8	10—3/4	7	13—3/4	7
17—1/4	9	13	7—1/2	12—3/4	9	10—1/2	8—3/4	12—1/4	10
18	10—1/2	13	7	12—1/2	7—1/2	11—1/2	7—1/2	10—1/2	8—3/4
16—3/4	9—1/2	11	8	12—3/4	7	11—1/4	8—1/2	13—1/2	7—1/2
16—3/4		11—1/2	7	12	7—1/2	12—1/4	7—3/4	13—3/4	6—1/2
		11—1/4		11—3/4	7—1/2	15	7—3/4	14—3/4	8—1/2
Weight, 702 mg.		10—3/4		13	7	10—1/2		12—1/4	9
17	11—1/2	12—1/2		12—3/4		11—3/4		12—1/2	8—3/4
16—3/4	9—1/2	12—3/4		11—3/4		12—1/4		11—1/4	
15—1/2	10	11—1/2		13		10—3/4		11—3/4	
17	11	11—1/2		12—1/2		11—3/4		12—1/2	
17	10	12—3/4		11—1/2		11—3/4			
16—1/2	9—1/2			13		11			
16—1/2	11			13		11—1/4			
16—1/2	9—1/2			12					
Mean: Strong Cobb—16.9		Mean: Strong Cobb—12.1				Mean: Strong Cobb—12.1			
Mean: Monsanto—10.1		Mean: Monsanto—7.4				Mean: Monsanto—7.9			
Ratio: Strong Cobb/Monsanto—1.67/1.00		Ratio: Strong Cobb/Monsanto—1.64/1.00				Ratio: Strong Cobb/Monsanto—1.53/1.00			

* Received August 11, 1954, from Strong, Cobb and Company, Inc., Cleveland, Ohio.

† Designer of the Strong Cobb Hardness Tester.

The assistance of Doctor George M. Naimark in writing this paper is gratefully acknowledged.

TABLE II.—TABLET HARDNESS MEASURED WITH STRONG COBB AND MONSANTO TESTERS

Acetylcarbromal; Standard Cup, 5/16-in. Die; Thickness, 0.276 in. Weight, 338–344 mg.			Digitalis Tablet; Standard Cup, 5/16-in. Die; Thickness, 0.196 in. Weight, 190–196 mg.			Calcium Carbonate, Magnesium Oxide; Semi-deep Cup, 7/32-in. Die; Thickness, 0.391 in.			
						Weight, 936–950 mg.		Weight, 952–972 mg.	
Strong Cobb No. 218	Strong Cobb No. 219	Monsanto No. 2	Strong Cobb No. 218	Strong Cobb No. 219	Monsanto No. 2	Strong Cobb No. 216	Monsanto No. 2	Strong Cobb No. 216	Monsanto No. 2
6—1/2	6—3/4	3—1/2	4	4—1/4	2—3/4	13—1/4	11	14—3/4	9
5—1/4	5—1/2	3—3/4	4	4—1/4	2—3/4	14	10	16—1/2	11
5—3/4	6—1/2	2—3/4	3—3/4	4	2—1/4	15	7	13—1/2	11
6—3/4	6—1/2	4	3—1/2	4	2	17	9	15	9
5—3/4	6—1/4	4	4	4	2	13—1/2	10—3/4	14—3/4	10
6—1/2	7—1/4	3—1/4	4—1/4	3—3/4	2—1/4	15	11	15	10—1/2
5—1/2	7—1/2	3—3/4	4—1/4	4—1/4	2—1/4	16		13—3/4	11
5—1/2	7—1/2	3—1/4	3—3/4	3—3/4	1—3/4	13—1/4		15—1/2	
7—1/2	6	4	4—1/4	4	2—1/4	13—1/4		14	
5	6	3—3/4	3—3/4	3—3/4	2			14—3/4	
6—1/2	6—1/2	4—1/4	3—3/4	3—3/4	2—1/4			13	
7	5	3	4	4—1/4	2—1/4			14—1/2	
6	6—1/2	3—1/4	3—3/4	4	2—1/4			14—3/4	
6—3/4	6	3—1/2	4	4	2—1/2			16—1/4	
6—1/2	5	4	3—3/4	3—3/4				15—1/2	
5—1/2	6—1/2	3—3/4	3—3/4	4—1/4				16—1/2	
								13—3/4	
								14—1/2	
								15—1/2	
								14—1/4	
Mean: Strong Cobb—6.1 Mean: Monsanto—3.6 Ratio: Strong Cobb/ Monsanto—1.69/1.00			Mean: Strong Cobb—4.00 Mean: Monsanto—2.25 Ratio: Strong Cobb/ Monsanto—1.77/1.00			Mean: Strong Cobb—14.3 Mean: Monsanto—9.9 Ratio: Strong Cobb/Monsanto—1.45/ 1.00			

TABLE III.—TABLET HARDNESS MEASURED WITH STRONG COBB AND MONSANTO TESTERS

Ferrous Sulfate, Quinine Sulfate; Deep Cup, 5/16-in. Die; Thickness, 0.265 in.				Ferrous Sulfate, Cobalt Sulfate; Deep Cup, 11/32-in. Die; Thickness, 0.265 in.		Aspirin Compound; Standard Cup, 11/32-in. Die; Thickness, 0.214 in.			
Weight, 200–209 mg.		Weight, 210–216 mg.		Weight, 310–326 mg.		Weight, 440–448 mg.		Weight, 450–468 mg.	
Strong Cobb No. 214	Monsanto No. 2	Strong Cobb No. 216	Monsanto No. 2	Strong Cobb No. 214	Monsanto No. 2	Strong Cobb No. 216	Monsanto No. 2	Strong Cobb No. 214	Monsanto No. 2
12	6—1/4	14—1/4	9	10—1/4	7	12—1/4	7—1/2	11—1/2	7
10—1/2	6—1/2	13	9—1/4	11	6	10—3/4	5—1/2	10—3/4	8
10—1/2	6—1/4	11—3/4	6—1/2	12	8	10—1/2	4—1/2	13—1/2	8
10—3/4	5—1/2	14	8	12	8	10—1/2	8	13—1/2	7
10—3/4	8—1/2	11—1/2	6	11—3/4	6—1/2	7	6—1/2	12	6—1/2
10—3/4	5—1/2	14	8—3/4	10—1/2	7—1/2			10—1/2	10—1/2
10—3/4	6	13—1/2	8—1/2	11	6	No. 217		13	5—1/2
10—1/2	9	11—1/2	8—3/4	11—3/4	8			13—3/4	8—1/2
10—3/4	6—1/2	15	7	11	7—1/2	10—1/2		13—3/4	7—3/4
11—1/4	5	12—1/2	9	12	6	13—3/4		12—1/2	7—3/4
12—1/2	8	12—1/2	9—1/4	11—1/4	7—1/2	10—3/4		7	8
11—3/4		14	6—1/2	10—3/4	7—1/2	12—1/4		13	8—1/4
		14	9—1/4	10—1/2	6—1/2			7	9—1/2
		16—1/2	8	10—1/2	7—1/2			12	
		14—1/2		12	7			9—1/2	
		15—1/4		11—1/4	6—3/4			11—1/4	
				12	7			11—1/4	
					8			10	
					6—1/2			11—3/4	
					9—1/2			12—1/2	
					9			11	
					8				
					7—1/2				
					7				
Mean: Strong Cobb—12.5 Mean: Monsanto—7.4 Ratio: Strong Cobb/Monsanto—1.70/1.00				Mean: Strong Cobb —11.3 Mean: Monsanto— 7.3 Ratio: Strong Cobb/ Monsanto— 1.54/1.00		Mean: Strong Cobb—11.3 Mean: Monsanto—7.4 Ratio: Strong Cobb/Monsanto— 1.56/1.00			

On the other hand, sodium bicarbonate tablets, 10-grain, of uniform size and weight were remarkably uniform in hardness. Figure 1 shows that slight variation in size (0.210–0.212 in.) and weight (690–708 mg.) did not significantly modify tablet hardness. Eight other tablet formulations were found to fulfill the necessary experimental conditions.

Fig. 1.—Tablets calcium carbonate, 10 grain. Strong Cobb Tester

EXPERIMENTAL

Tablets of all nine types were segregated into groups containing tablets of uniform thickness and weight. Hardness was determined with the Strong Cobb and Monsanto hardness testers on a number of tablets from each group. In some cases tablets were tested using more than one instrument of each type to obtain data on the variation between testers of the same type. Results are shown in detail in Table I.

DISCUSSION

The most significant finding of this experiment was the constant ratio of hardness results obtained from both types of hardness testers with various tablet formulations. As the data in Tables I, II, and III illustrate, with uniformly hard tablets, the Strong Cobb tester will, on the average, yield results 1.6 times the value given by the Monsanto tester. It is also seen that a given instrument gives reproducible results and that multiple instruments of both types check each other quite closely. The hardness range of the Strong Cobb tester (0–30 Kg.) is greater than that of the Monsanto tester (0–20 Kg.). However, the consistently lower readings obtained on the Monsanto instrument result in the usable hardness ranges being approximately the same.

The two instruments do not accommodate tablets of the same dimensions; a $^1/_2$-in. tablet is the largest measurable on the Monsanto hardness tester while $^3/_4$-in. is the largest tablet size accommodated by the Strong Cobb hardness tester.

SUMMARY

1. Tablets of uniform characteristics must be used to correlate hardness testers.

2. On the average, the Strong Cobb tester gives results 1.6 times those of the Monsanto hardness tester.

3. Single instruments of either type will give reproducible results.

4. Multiple instruments of both types check each other quite closely.

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Sorbic Acid as a Fungistatic Agent for Certain Pharmaceutical Preparations*

By D. D. PULS,† L. F. LINDGREN,‡ and F. P. COSGROVE§

A comparison of the fungistatic property of sorbic acid and benzoic acid was undertaken to determine their efficacy against common mold contaminants and as a preservative in certain pharmaceutical preparations that are susceptible to mold growth. Sorbic acid was found to be superior to benzoic acid as a fungistatic agent.

IT HAS BEEN SHOWN by Gooding (1) that certain of the α-β-unsaturated fatty acids are good fungistatic agents for foods. Sorbic acid (CH_3—CH=CH—CH=CHCOOH)[1] is a particularly well suited substance for this purpose. Smith and Rollin (2) have found sorbic acid to be markedly superior to sodium benzoate as a fungistatic agent for cheese products which are very susceptible to mold spoilage.

Deuel, *et al.* (3), have also reported that sorbic acid is remarkably low in toxicity and is metabolized in exactly the same manner as the naturally occurring saturated fatty acid, caproic, both yielding carbon dioxide and water as end products (4).

Our study was undertaken to compare the fungistatic property of sorbic acid with that of

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[1] Supplied by Carbon & Carbide Company, New York.

benzoic acid against common mold contaminants and to determine the efficacy of sorbic acid as a preservative in certain pharmaceutical preparations that are susceptible to mold growth.

METHODS

A Comparison of the Fungistatic Action of Sorbic Acid with That of Benzoic Acid.—To compare the fungistatic property of sorbic acid with benzoic acid, percentage concentrations of 0.05, 0.075, 0.10, 0.20, and 0.40 of each drug were prepared and tested against *Aspergillus niger*,[2] and an unidentified *Penicillium species* F.P.C.,[2] using the agar-disk-plate method. All tests were performed in triplicate. Distilled water was used as a control.

Plates were prepared by distributing one loopful of spores in 5 ml. of nutrient broth, adding 0.5 ml. of the inoculated broth to 200 ml. of agar-malt extract,[3] previously cooled to 45°, and then pouring approximately 15 ml. of this mixture into each petri dish.

Paper disks, 12 mm. in diameter, were saturated with the test solutions, superimposed on the media, and the plates incubated for forty hours at 30°. Results expressed as zones of inhibition appear in Tables I and II.

TABLE I.—ZONES OF INHIBITION OF ASPERGILLUS NIGER

Percentage of Fungistatic Agent	Zones of Inhibition in mm. Sorbic Acid	Benzoic Acid
0.00 (no inhibition)		
0.05	[a]	[a]
0.075	[a]	[a]
0.10	2[c]	[a]
0.20	2,[b] 6[c]	[a]
0.40	5,[b] 9[c]	3[c]

[a] No inhibition.
[b] Complete inhibition.
[c] Partial, or complete plus partial inhibition.

TABLE II.—ZONES OF INHIBITION OF PENICILLIUM SPECIES

Percentage of Fungistatic Agent	Zones of Inhibition in mm. Sorbic Acid	Benzoic Acid
0.00 (no inhibition)		
0.05	4[c]	[a]
0.075	5[c]	[a]
0.10	8	1[c]
0.20	10,[b] 12[c]	3[c]
0.40	13,[b] 18[c]	6,[b] 8[c]

[a] No inhibition.
[b] Complete inhibition.
[c] Partial, or complete plus partial inhibition.

Effectiveness of Sorbic Acid as a Pharmaceutical Preservative.—In order to determine the effectiveness of sorbic acid in preserving certain pharmaceutical preparations, varying amounts of the drug were incorporated into the following samples: 35.0% acacia mucilage, 6.0% tragacanth mucilage, 1.0% guar gum[4] solution, and 26.0% sucrose solution. The preservatives were dissolved in distilled water with the aid of heat. All samples were prepared in triplicate, stored at 5, 25, and 37° for thirty days, and then tested for mold growth using the agar plate method.

Official samples of acacia mucilage and tragacanth mucilage as well as controls without any preservative were prepared and treated in the same manner.

The preparations were observed once a day for the first seven days and then once a week for the remainder of the storage period. During the storage time, each container was open several times in order to subject the contents to possible contamination from the atmosphere.

All the samples were tested for mold count by the dilution method with the exception of the samples of tragacanth mucilage. Due to the viscous nature of the latter preparations, only surface streaks were made in order to determine the presence of mold growth. Results are given in Table III.

DISCUSSION

Because sorbic acid is soluble only to the extent of 1:500 at 20°, crystallization occurred in some of the samples. Since concentrations as low as 0.05% appear to preserve the samples tested, the factor of solubility does not appear to be a serious one.

Most of the samples tested showed very little color change during the storage period. Samples of acacia containing 0.2% sorbic acid and stored at 37° showed some discoloration. The other acacia samples compared favorably in color and odor with the official preparation.

Melnick, *et al.* (5), state that sorbic acid may act as a fungistatic agent by interfering with the dehydrogenase systems of various molds and recommend that reasonable care be taken during the preparation of any sample protected with this agent to avoid gross contamination.

SUMMARY

1. A study of the fungistatic property of sorbic acid is reported.

2. Sorbic acid is superior to benzoic acid as a fungistatic agent against *Aspergillus niger* and an unidentified *Penicillium* species when tested by the agar-disk-plate method.

3. Sorbic acid concentrations as low as 0.05 per cent preserve 35.0 per cent acacia mucilage, 6.0 per cent tragacanth mucilage, 1.0 per cent guar gum solution, and 26.0 per cent sucrose solution when stored at 5, 25, and 37° for thirty days. Species of *Alternaria*, *Penicillium*, *Aspergillus*, and *Pseudo-yeast* were identified in the controls and in some samples containing less than 0.05 per cent of preservative.

[2] Obtained from the Bacteriology Department, University of Nebraska, Lincoln.
[3] 1.5 per cent Difco malt extract broth and 2.0 per cent Difco Bacto Agar, Difco Laboratories, Incorporated, Detroit, Michigan.
[4] Jaguar gum A-20-B, supplied by Stein, Hall & Co., New York, N. Y.

TABLE III.—THE EFFECT OF SORBIC ACID ON THE MOLD GROWTH IN CERTAIN PHARMACEUTICAL PREPARATIONS

Percentage of Fungistatic Agent	Storage Condition	Average Number of Mold Colonies per ml. of Sample[a]			
		Acacia Mucilage	Tragacanth Mucilage[b]	1% Guar Gum Solution	26% Sucrose Solution
Control	37°	4067	Growth[c]	966	470
0.20 Benzoic		33	No growth	Not tested	Not tested
0.01 Sorbic Acid		19	Not tested	35	0
0.05 Sorbic Acid		0	No growth	0	0
0.10 Sorbic Acid		0	No growth	0	0
0.20 Sorbic Acid		0	No growth	0	0
Control	25°	533	Growth	5566	157
0.20 Benzoic Acid		0	No growth	Not tested	Not tested
0.01 Sorbic Acid		33	Not tested	8800	385
0.05 Sorbic Acid		0	No growth	0	0
0.10 Sorbic Acid		0	No growth	0	0
0.20 Sorbic Acid		0	No growth	0	C[d]
Control	5°	133	Growth	3666	80
0.20 Benzoic Acid		0	No growth	Not tested	Not tested
0.01 Sorbic Acid		22	Not tested	66	0
0.05 Sorbic Acid		0	No growth	0	0
0.10 Sorbic Acid		0	No growth	0	C[d]
0.20 Sorbic Acid		C[d]	No growth	C[d]	C[d]

[a] Identified as *Alternaria sp.*, *Penicillium sp.*, *Aspergillus niger*, and *Pseudo-yeast*.
[b] Only surface streaks of samples were made.
[c] No growth in one of the three control samples stored at 37°.
[d] Sorbic acid crystallized.

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Composition of the Volatile Oil from the Foliage and Terminal Branches of Western Juniper*

By MICHAEL D. FAHEY and E. F. KURTH

The volatile oil obtained by steam distillation of the foliage and terminal branches of western juniper in 1.8 per cent yield was found to contain α-pinene 10–12 per cent; a mixture of β-phellandrene, dipentene, and p-cymene 10 per cent; sabinene 4 per cent; terpinolene and α-terpinene 3–5 per cent; terpinen-4-ol 6–8 per cent; d-borneol, free and combined as borneol acetate, 22–25 per cent; cadinene 20–25 per cent; acetic acid, free and combined as borneol acetate, 4–5 per cent; phenols 1.0 per cent; traces of aldehydes; and residue and losses 16–18 per cent.

AN ANALYSIS of the volatile oil from the foliage and terminal branches of western juniper (*Juniperus occidentalis*, Hooker) was made by Lynn and Fischer on an oil which had been rectified by steam distillation from a three-year-old resinified sample (1). An analysis on a freshly collected sample of this oil gave a composition different from that reported by Lynn and Fischer and distinctly different from the wood oil of this species (2).

Comparison of the composition of the fresh oil sample used in this investigation with the partially resinified sample analyzed by Lynn and Fischer showed that the fresh sample contained α-pinene, sabinene, terpinolene, terpinen-4-ol, dipentene, cadinene, β-phellandrene, and possibly γ-terpinene in addition to the components reported present in the resinified oil. The presence of camphene, which Lynn and Fischer thought to be present but were unable to identify because of insufficient material, could not be confirmed. It appears that the fraction they believed to be camphene was actually sabinene.

* Received August 19, 1954, from the Department of Chemistry, Oregon State College, and The Oregon Forest Products Laboratory, Corvallis, Ore.
A condensation of the thesis presented by Michael D. Fahey in partial fulfillment of the requirements for the Master of Science degree in Chemistry at Oregon State College, June, 1954.

EXPERIMENTAL

Material.—Fresh foliage samples for this study were obtained in January and May, 1953, in central Oregon between Sisters and Bend, where

there are nearly continuous stands of the western juniper. The samples consisted of terminal branches up to 1/4 in. in diameter, and the accompanying foliage. To insure representative samples, the material was obtained from 40 trees, varying from saplings to trees 3 ft. in diameter. About 5–10 lb. of material were cut from each tree with pruning shears. This was then comminuted in an ensilage cutter, thoroughly mixed, and placed in covered containers.

Oil-Recovery.—Quantitative determination of the oil content was made by steam distillation of 100–125 Gm. samples in a Clevenger apparatus for a period of sixteen to twenty-two hours. Shorter periods did not recover all of the oil and no noticeable increase in the oil content was observed for distillation periods beyond twenty-two hours. The oil yield for 16 determinations varied between 1.53 and 2.72% and averaged 2.05% calculated on the oven-dry weight of the samples. The moisture content of the material at the time of distillation averaged 42.7%.

For the isolation of a volume of oil suitable for analysis, batch distillations of the foliage were carried out in a still that had a capacity of 12–14 lb. of fresh material. Steam was conducted into the base of the still and into a jacket surrounding the still pot. Pressure of the steam in the still could be regulated from 0 to 20 p.s.i. by adjustment of a valve in the condenser system. At atmospheric pressure the ratio of oil recovered to aqueous distillate was 43 ml. oil and 20 L. water; at 8 p.s.i. steam pressure the ratio was 45 ml. oil to 16 L. water; and at 15 p.s.i. steam pressure the ratio was 46 ml. oil to 12 L. of water. A further increase in steam pressure caused the oil to darken and change odor. Therefore, the conditions adopted for recovery of the oil from the comminuted foliage were a steam distillation pressure of 14–16 p.s.i. and a distillation rate of 4 L. per hour for a total of three hours. About one quarter of the total oil yield was dispersed in the aqueous layer of the distillate and was recovered by extraction with petroleum ether. Foliage with a calculated dry weight of 133.2 lb. gave 2.37 lb. oil, or a yield of 1.8%.

Properties of the Oil.—The oil mechanically separated from the distillate had a light yellow color, whereas the oil extracted with petroleum ether from the aqueous condensate was reddish brown. Both oil fractions had the same fragrant odor, typical of the juniper foliage. Density, d_{20}^{20}, of the total oil was 0.9197, refractive index n_D^{20} was 1.4783, and the specific rotation was $[\alpha]_D^{20}$ + 23.68°. Acid number was 4.96. Saponification numbers before and after acetylation were 46.4 and 80.4. Initial distilling temperature was approximately 150°, with about one third of the oil distillable below 180° without decomposition at atmospheric pressure.

Aldehydes, Phenols, and Acids.—A 280-Gm. portion of the oil extracted with saturated neutral sodium sulfite solution gave 3.0 Gm. of aldehydes, or a yield of about 1.0%. Basic extractions gave 2.3 Gm. phenols, 0.82%, and 1.6 Gm. free acids, 0.57%. Saponification of the oil by refluxing for two hours with alcoholic sodium hydroxide solution gave 4.0% total acids, calculated as acetic acid, and 1.0% total phenols. The presence of acetic acid was established by the isolation of potassium acetate, m. p. 290° and the formation of the *p*-bromo-phenacyl ester, m. p. 84–85°. The phenol fraction of the oil had a distilling range of 184–234°.

α-Pinene.—The neutral portion of the saponified oil was fractionally distilled in a 36-in. Stedman column, equipped with a heating jacket and a constant-reflux-ratio head. The fractions distilling between 60 and 68° at 29 mm. Hg amounted to 10–12% of the total oil and indicated the presence of α-pinene. A nitrosochloride derivative prepared by Wallach's method had 113–114° m. p. The nitrosochloride converted to the nitrolpiperidine had m. p. of 119°. Optical rotation measurements, $[\alpha]_D^{25}$ +26.2–51°, indicated a mixture of *d*- and *dl*-α-pinene. Tests for fenchene and thujene gave negative results.

Sabinene.—The fractions distilling between 68 and 75° at 29 mm. Hg had a boiling range of 160–163° at atmospheric pressure, and amounted to 4% of the oil. Terpenes in this boiling range include camphene, β-pinene, and sabinene. The fractions were purified by redistillation over metallic sodium. Evidence for the absence of camphene was provided when the oil would not crystallize when cooled to −10° nor form a hydrochloride derivative. Conversion to the oxidation product by Wallach's procedure (3) with potassium permanganate gave sabinenic acid, m. p. 59° and neutralization equivalent 187. It was concluded that only sabinene was present.

β-Phellandrene.—Fractions of the oil distilling between 75 and 80° at 28 mm. Hg amounted to 10–12%. Compounds included in this boiling range are the terpinenes, phellandrenes, dipentene, Δ^3-carene, and para-cymene. The absence of the terpinenes was indicated when no crystalline hydrochloride could be formed. Tests for Δ^3-carene through nitrosate and color reaction gave negative results. The presence of phellandrene was shown by the formation of a nitrosite derivative by Gaponenkov's procedure (4), m. p. 108–110° No adduct with maleic anhydride in ethyl ether could be isolated. This indicated the absence of α-phellandrene. Bromination by the method of Baeyer (5) produced a crystalline bromide, m. p. 116–118°. Lit. for m. p. of β-phellandrene tetrabromide was 118–119°.

Dipentene.—A fraction distilling between 78.5 and 80° at 28 mm. Hg and 175–177° atmospheric pressure formed a crystalline acid with maleic anhydride when refluxed for one hour in benzene. After recrystallization, the acid melted at 147°, which is the m. p. of the acid, $C_{14}H_{20}O_4$ obtained by Hultzsch with dipentene and maleic anhydride (9).

***p*-Cymene.**—The above fraction also indicated the presence of *p*-cymene through the products obtained by alkaline permanganate oxidation according to Wallach's procedure for terpenes (6). *p*-Hydroxyisopropyl benzoic acid, m.p. 156–157°, neutralization equivalent 183, was isolated from the oxidation mixture. This was converted to *p*-isopropenyl benzoic acid by heating with dilute hydrochloric acid, m. p. 160–161°.

Terpinolene and γ-Terpinene.—The fractions distilling between 81 and 89° at 28 mm. Hg corresponded to the properties of terpinolene and γ-terpinene. Bromination by the method of Baeyer (5) produced terpinolene tetrabromide, m. p. 115–

116. Oxidation with alkaline permanganate (6) gave two neutral products. They were separated by differences in solubility in ethanol. The first product, which was quite soluble in ethanol, was recrystallized from ethyl acetate and melted at 148–149° with gas evolution. This is the m. p. of *p*-menthane 1, 2, 4, 8 tetrol, the corresponding oxidation product of terpinolene. The second product was sparingly soluble in ethanol and melted 239–240°. This is the m. p. of *p*-menthane 1, 2,4,5 tetrol, the oxidation product of γ-terpinene. The oil fractions amounted to 3–5% of the total oil.

Terpinen-4-ol.—Fractions of the oil distilling between 85 and 90° at 9.4 mm. Hg indicated the presence of terpene alcohols. A fraction distilled at 85.5–87°, 9.4 mm. Hg, was treated for the formation of nitrosochlorides. White crystals melting at 115–116° separated. This is the reported m. p. of terpinen-4-ol-nitrosochloride (7). It was converted to terpinen-4-ol-nitrolpiperidine, m. p. 171–173°. About 6–8% of the oil was estimated to be terpinen-4-ol.

Borneol.—The distillate reaching the head of the column solidified at around 88° at 9.4 mm. Hg. Therefore, the Stedman column was replaced with an 8-in. insulated Vigreux column and the distillation completed at 1.2 mm. Hg. The crystalline solid was identified as borneol by formation of the phenyl urethane, m. p. 138°, formation of its 3,5-dinitrobenzoate, m. p. 155–156°, and its melting point 202–204°. The free and combined borneol content was estimated at 22–25%.

Cadinene.—About 25% of the oil distilled above 70° at 1.0 mm. Hg and was in the boiling range of the sesquiterpenes and their alcohol derivatives. Tests for alcohols gave negative results. Treatment with a cold, saturated solution of hydrogen chloride in glacial acetic acid produced cadinene dihydrochloride, m. p. 116–117°. Dehydrogenation by Ruzicka's procedure (8) with sulfur gave a 30% yield of cadalene. This compound was identified by the formation of its picrate, m. p. 114–115°.

Residue and Losses.—Residue and losses amounted to 16–18%; about half was a dark, sticky mass, which was probably derived from some of the easily polymerized terpenes such as the phellandrenes. In addition to cadinene other sesquiterpenes may be present in the high boiling fractions, but cedrene, which is a major component of the volatile oil from the wood of western juniper, was not found present.

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The Microscopic Structure of Lemongrass Leaves*

By SHAFIK I. BALBAA† and CARL H. JOHNSON

The volatile oil of lemongrass was found in the blade to occur in rows of cells located immediately below the bands of bulliform cells. In the sheath the rows of oil cells are embedded in the central part of the fundamental parenchyma. Such cells also occur in the joint triangles. No oil cells occur in the ligule, the auricles, or the culm. The oil cells, when freed of oil, are similar in appearance to some adjacent parenchyma but can easily be distinguished by the effect of phloroglucin-hydrochloric acid on the walls. The detailed structure of the parts of the leaf is illustrated and described.

IN ORDER TO STUDY the optimum conditions for growth and the production of volatile oil in lemongrass it was deemed necessary to ascertain the position of the oil in the leaf. The only work referring to the location of the oil is that of Jones and Arrillaga (1), who merely stated that it existed in oil glands and secretory cells in the leaf, without giving more details. Also, there is no reference in the literature to work on the microscopic structure of lemongrass or any related species, although other grasses of dissimilar appearance have been described (2–5). Hence this study of the structure of the leaf in relation to the oil-containing cells was made.

The material used in the investigation was grown in the Medicinal Plant Garden of the College. The species was *Cymbopogon flexuosus* (Nees) Stapf, the source of the East Indian lemongrass oil of commerce, this identification being confirmed by Dr. Joseph R. Swallen, U. S. National Museum, Smithsonian Institution, Washington, D. C. The organs examined were the vegetative lower leaves as distinguished from those arising from the flowering culm produced late in the season. The material was either fresh or preserved in seventy per cent alcohol contain-

* Received October 13, 1954, from the College of Pharmacy, University of Florida, Gainsville.
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An Edwin Leigh Newcomb Memorial Award Essay.
Abstracted from a dissertation submitted by Shafik I. Balbaa to the Graduate Council of the University of Florida in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ing five per cent of glycerin. The drawings were prepared with the aid of a camera lucida.

GENERAL MORPHOLOGY

In general appearance, the leaves of lemongrass are like those of other grasses that grow in large tufts. They are borne on culms in alternating ranks. Each leaf consists of two parts, the split sheath and the blade, connected at the blade joint (Fig. 1). The inner surface of the blade joint, or throat, is limited basally by a membranous appendage called the ligule. The region forming the back or outer surface of the blade joint is the collar and is composed of two somewhat thickened triangular areas, called joint triangles, that differ in color and texture from the surrounding tissue. Projecting from the sheath margin below the blade joint are two appendages known as the auricles.

The blades are linear-lanceolate, tapering toward the apex and often ending in a long fine point. Toward the base, the blade narrows somewhat to the sheath. The length and width of the blades vary greatly, being as much as 130 cm. long and 3.5 cm. wide. The leaf is thin and somewhat rough to the touch due to the presence of spines on both surfaces. The margin of the blade appears very finely serrate from teeth formed by stiff ascending bristles. On the upper surface of the blade in its lower part, scattered long silky hairs are visible.

The sheath may be up to 50 cm. long and 8 cm. broad. Like the lamina, it contains many parallel veins, which are largest in the thicker center, gradually becoming smaller toward the edges. There is no midrib except at the top, where the midrib of the blade extends downward. The sheath is inserted in the tissue of the node (Fig. 1*D*), forming an even line around the culm, with the overlapping edge of the sheath projecting slightly down the stalk. The region immediately above the line of insertion is called the sheath base.

The ligule (Fig. 1*B*), when young, is translucent and hyaline, with the free upper margin entire and slightly wavy later, it becomes dry and may appear irregularly incised, especially at the middle. The average height of the ligule is 3 mm. The two ends

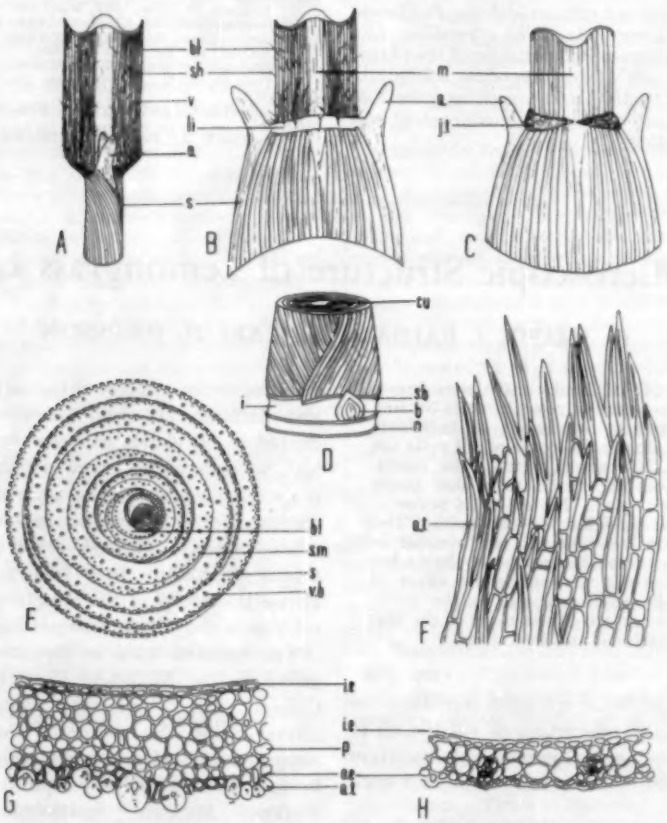

Fig. 1.—Base of shoot, blade joint, and adjacent parts. *A*. Blade joint of a detached leaf. *B*. Inner surface of opened blade joint. *C*. Outer surface of opened blade joint. *D*. Part of culm with leaf bases. *E*. Cross section of shoot. *F*. Outer epidermis of ligule. *G*. Cross section of ligule. *H*. Cross section of auricle. *a*, auricle; *a.t*, adnate trichome; *b*, bud; *bl*, blade; *cu*, culm; *i*, internode; *i.e*, inner epidermis; *j.t*, joint triangle; *li*, ligule; *m*, midrib; *n*, node; *o.e*, outer epidermis; *p*, parenchyma; *s*, sheath; *s.b*, sheath base; *s.h*, silky hair; *s.m*, sheath margin; *v*, vein; *v.b*, vascular bundle.
A–C, ×1.1. *D*, ×1.2. *E*, ×3.2. *F–H*, ×190.

normally terminate at the edge of the leaf where the auricles arise.

The auricles (Fig. 1*A*, *B*, *C*) form more or less oblong structures which are translucent and asymmetrical. Each leaf sheath has two of them overlapping, of which the outer is smaller and shorter than the inner. Their tips project prominently above the upper margin of the joint triangles.

The culm, which arises from a short oblique annulate rhizome, varies greatly in size depending on its stage of development. It is short and inconspicuous during the early part of the season, being enclosed by the lower part of the leaf sheaths. Later, it elongates rapidly, and may end in an inflorescence if conditions are favorable. Above each node and covered by the sheath base is a bud which appears as a conchate appressed structure sunken in the culm (Fig. 1*D*). Generally, the length and thickness of the internodes decrease from the middle part of the culm acropetally and basipetally.

HISTOLOGY

The tissues of the blade are continuous with those of the sheath and in general show a similar structure. The differences are consistent with the chief role of the blade as an organ of photosynthesis.

The Vascular Bundles.—The parallel vascular bundles in the leaf blade are of three orders or ranks: Large rhomboidal, medium large oval, and small round bundles. In the cross section of the leaf (Fig. 2), they appear as a row of isolated units extending from margin to margin. Usually groups of one to four small bundles alternate with the large or medium large bundles. The small bundles, and usually the medium bundles, lie closer to the lower epidermis, whereas the large bundles extend to the upper epidermis. In the region of the midrib, however, a thick, triangular or crescent-shaped layer of colorless parenchyma is interposed between the bundles and the upper epidermis, pressing the vascu-

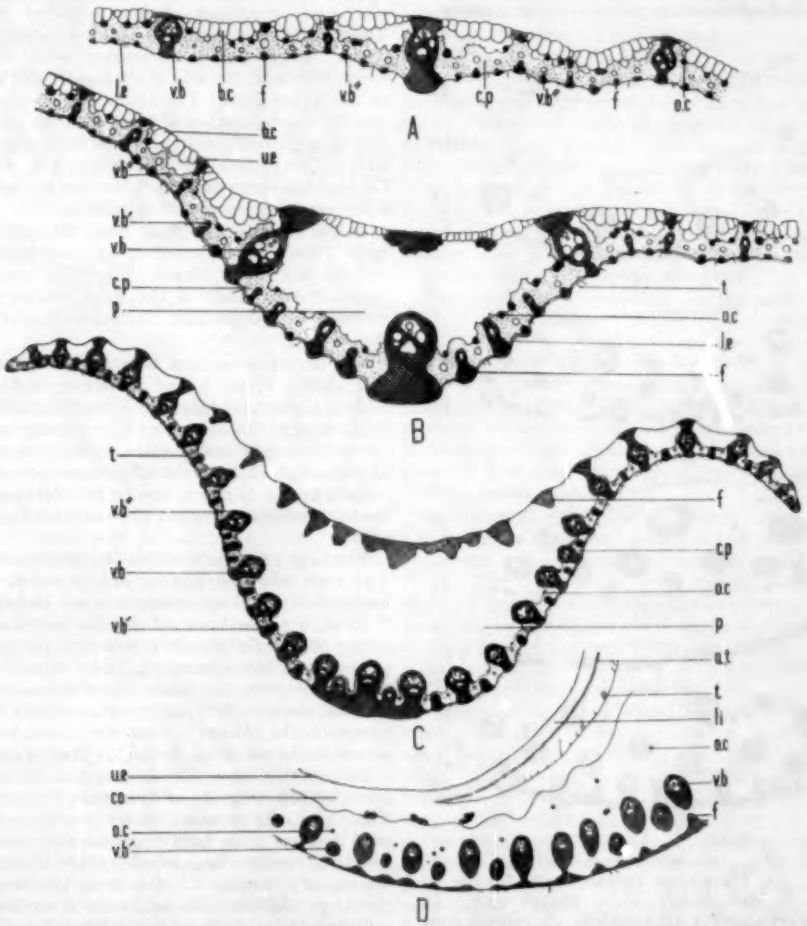

Fig. 2.—Diagrammatic cross sections of the blade. *A*. Upper third, including midrib. *B*. Middle part, including midrib. *C*. Basal region. *D*. Blade joint and ligule. *a.t*, adnate trichome; *b.c*, bulliform cell; *co*, collenchyma; *c.p*, chlorophyll-bearing parenchyma; *f*, fibers; *li*, ligule; *l.e*, lower epidermis; *o.c*, oil cell; *p*, parenchyma; *t*, trichomes; *u.e*, upper epidermis; *v.b*, large vascular bundle; *v.b'*, medium large vascular bundle; *v.b''*, small vascular bundle.
A, *B*, and *D*, ×48. *C*, ×21.

lar tissue downward. In this region, the parenchyma underlying the upper epidermis is reinforced by groups of sclerenchymatous fibers opposite the large bundles.

In the sheath (Fig. 3) a cross section through the central part shows vascular bundles in the fundamental parenchyma, but lying closest to the outer epidermis; bundles of two different orders alternate with each other here, the large rhomboidal and the medium large oval bundles. The base of the sheath has more bundles which are arranged in more or less definite tangential bands, with each band containing bundles of a single magnitude, the ones closest to the inner epidermis being the largest and most widely spaced. The upper end of the sheath becomes narrower and thicker before it merges into the blade, thereby crowding the vascular bundles together.

In the auricles (Fig. 1*H*) are two or three layers of parenchymatous cells longitudinally traversed by numerous vascular bundles lying closer to the outer surface and infrequently connected transversely.

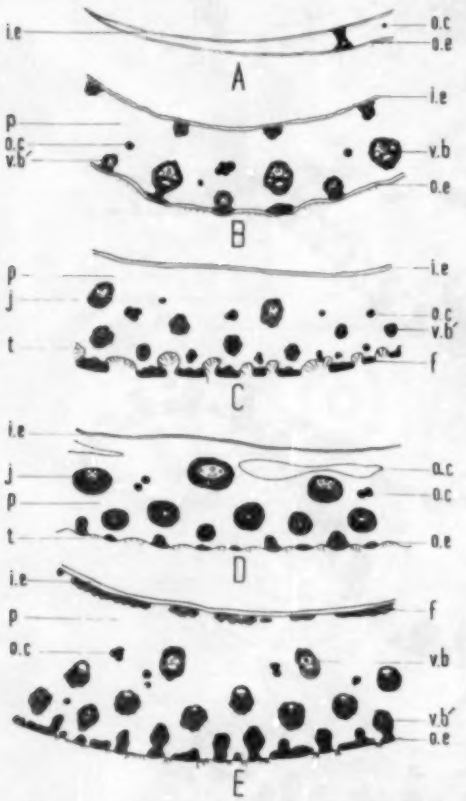

Fig. 3.—Diagrammatic cross sections of the sheath. *A*. Margin of sheath. *B*. Middle of sheath. *C*. Immediately above sheath base. *D*. Sheath base showing air cavities. *E*. Sheath base where it joins culm. *a.c*, air cavity; *f*, fibers; *i.e*, inner epidermis; *j*, sclerenchyma jacket; *o.c*, oil cell; *o.e*, outer epidermis; *p*, parenchyma; *t*, trichomes; *v.b*, large vascular bundle; *v.b'*, medium large vascular bundle.
A, ×54. *B–E*, ×24.

There is little or no vascular tissue in the ligule.

In the culm the veins follow a longitudinal course except in the nodes where many branch or bend abruptly toward the periphery. The bundles (Fig. 4) are not arranged in a simple ring, but lie scattered, except in the center, leaving the section with a central pith. The number of bundles increases toward the periphery, whereas their size gradually decreases.

In general, all the veins (Figs. 4–7) are structurally similar. The phloem forms an oval to kidney shaped mass of tissue with the convex side toward the xylem arc. It consists of sieve tubes and companion cells. The protophloem lies in a narrow band nearest to the lower side of the leaf; sometimes its cells show somewhat swollen and disorganized walls.

The xylem consists of protoxylem, metaxylem, and accompanying parenchyma. The protoxylem is made up of annular and spiral vessels which may appear in the larger veins as a protoxylem lacuna containing remnants of the ruptured elements. The metaxylem of the large bundles consists usually of two large vessels; sometimes when there are three, two lie at one side of the bundle and the third at the other side. The tissue between the large vessels is composed of a band of small pitted and reticulate elements most of which are narrow vessels having the perforations on oblique end walls. In the medium large and small bundles there are only a few relatively large pitted vessels.

In the culm (Fig. 4) the tissue between the two large vessels is composed of lignified parenchyma interspersed with narrow reticulate and pitted vessels. Also, some of the large vessels are surrounded by tangentially flattened lignified parenchyma cells.

The large and medium large bundles of the blade and sheath (Figs. 5 and 6) are embedded in a schlerenchymatous jacket which is incomplete in the medium large bundles where it frequently surrounds the phloem only and not the xylem. In the base of the sheath (Fig. 7) the sclerenchymatous jackets enclosing the bundles are better developed and thicker, especially toward the outer surface of the sheath.

The large vascular bundles of the blade and sheath with their sclerenchymatous jackets are surrounded by an additional conspicuous inner sheath (Figs. 5–7) of a single layer of thinner-walled lignified cells. Where the bundle is joined to the epidermis by a sclerenchyma band, the inner sheath becomes continuous with the band. In the medium large bundles, the inner sclerenchymatous sheath partially surrounds the phloem but not the xylem, while it is absent in the small bundles of the third order.

Outside this inner sclerenchymatous sheath is the outer sheath (Fig. 5) of thin-walled parenchyma. These parenchymatous cells are usually rounded or oval and are more easily recognizable around the small and medium large bundles of the blade. They are radially elongated and more or less irregular in the large bundles. In longitudinal section, these cells are easily distinguishable by being elongated in the direction of the long axis of the leaf. The cells of this outer sheath contain chloroplasts which are usually larger and less crowded than those of the surrounding chlorophyll-bearing parenchyma. The outer sheath around the larger bundles is interrupted

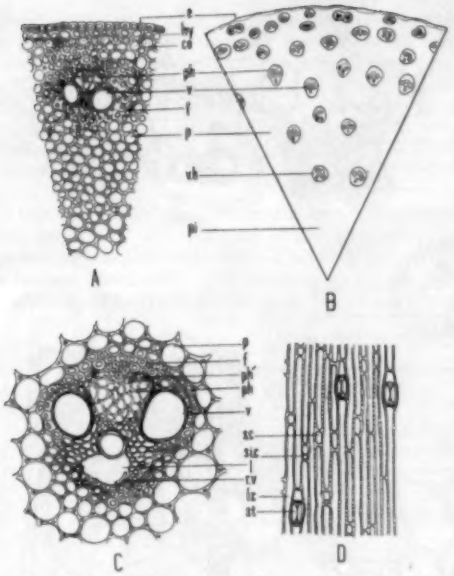

Fig. 4.—Structure of the culm. *A*. Cross section in peripheral part. *B*. Diagram of cross section of internode. *C*. Cross section of a central vascular bundle. *D*. Surface view of epidermis. *co*, cortex-like tissue; *e*, epidermis; *f*, fibers; *hy*, hypodermis; *l*, protoxylem lacuna; *l.c*, long cell; *p*, parenchyma; *ph*, phloem; *ph'*, protophloem; *pi*, pith; *r.v*, remains of vessel; *si.c*, silica cell; *s.c*, suberized short cell; *st*, stoma; *v*, metaxylem vessel; *v.b*, vascular bundle. *A*, *C* and *D*, ×160. *B*, ×16.

where they are joined to the epidermis by a sclerenchymatous band.

The Mechanical Tissue.—The blade and the sheath have a considerable amount of mechanical tissue (Figs. 2, 3) arranged in bands running the whole length of the leaf. They may be joined laterally to form very broad zones. The usual position of these bands is just within the epidermis above or below the large and medium vascular bundles and at the margin of the blade. In the larger bundles, the bands are continuous with the inner sclerenchymatous sheaths, and thus form girders to the bundles, joining these to each epidermis. The other vascular bundles are frequently joined to the lower epidermis only, with the upper bands separate from the bundle.

The fibers making up this tissue are either short or very long. They appear polygonal in cross section with varying diameters. They usually have lignified, thick pitted walls and narrow lumens, and end in a narrow acute tip.

The Parenchyma.—The chlorophyll tissue (Fig. 5) appears in transverse section as a mass of more or less isodiametric cells with small intercellular spaces. This tissue nearly fills the area between the vascular bundles of the blade. The cells contain chloroplasts that are usually smaller and denser than in the case of the outer bundle sheath. Between the chlorophyll-bearing cells and the bulliform epidermal cells described later, other parenchymatous cells occur that appear rounded or oval in cross section and are elongated in the direction of the long axis of the leaf.

The fundamental parenchyma cells of the leaf sheath (Fig. 6) have fewer chloroplasts than in the case of the blade. In the lower end, the parenchymatous cells near the inner epidermis are tangentially elongated and compressed; some show partial disintegration with the formation of tangentially elongated air cavities (Fig. 3). Occasional prismatic calcium oxalate crystals are found in the parenchyma cells of the sheath base.

In the ligule (Fig. 1*G*) the interior is made up entirely of 6 to 7 layers of parenchymatous cells which are rounded and comparatively thick-walled, especially near the inner epidermis. They are largest in the central portion of the ligule.

Next to the epidermis of the culm (Fig. 4*A*) are one to six rows of hypodermal cells with lignified thick walls. In longitudinal section they are long and sclerenchymatous. The continuity of this sclerenchymatous mantle, however, is broken by the occasional interruption of parenchymatous cells extending externally to a stoma.

The outer region of the parenchyma lying next to this hypodermis is composed of a few rows of thin-walled cells forming a narrow cortex-like tissue containing few chloroplasts. There is a gradual transition from this area to the cells which form the filler between the peripheral bundles. The parenchyma cells are progressively larger and become lignified toward the center of the culm. In cross section the cells are rounded or elliptical, whereas vertically they are somewhat elongated.

The Oil Cells.—Frequently the parenchymatous cells of the blade are replaced by the similar oil cells (Fig. 5) that form rows parallel to the long axis and between the vascular bundles. In the thin parts of the blade, the oil cells commonly lie beneath the bulliform epidermal cells and are surrounded on the other sides by chlorophyll-bearing parenchyma tissue. The rows of oil cells are usually long but occasionally are very short or infrequently a single isolated cell occurs.

In the cross section, they usually occur singly and occasionally in small groups of two to five, with the common walls between the cells usually flattened. The adjoining oil cells may be of different sizes. They are usually rounded or somewhat oval in shape, but in longitudinal sections they appear more or less rectangular, joining each other at their narrow ends.

The oil cells have thin suberized walls. They can be easily distinguished when free of volatile oil in sections mounted in phloroglucin and hydrochloric acid since the walls acquire a slight reddish yellow color and show high refraction. In the mature fresh leaves these cells are completely filled with a light yellow volatile oil. In the old or dry leaves where the oil resinifies, lumps of reddish-yellow resin occupy a part of the cell cavity. In young leaves some oil cells are filled with small oil droplets resembling an emulsion and are accompanied by fragments of protoplasm whereas in the same sections other oil cells are entirely filled with volatile oil. These observations seem to conform with the view of A. Leemann (6) on the formation of volatile oil in secretory cells.

Cross sections from the middle of the blade showed

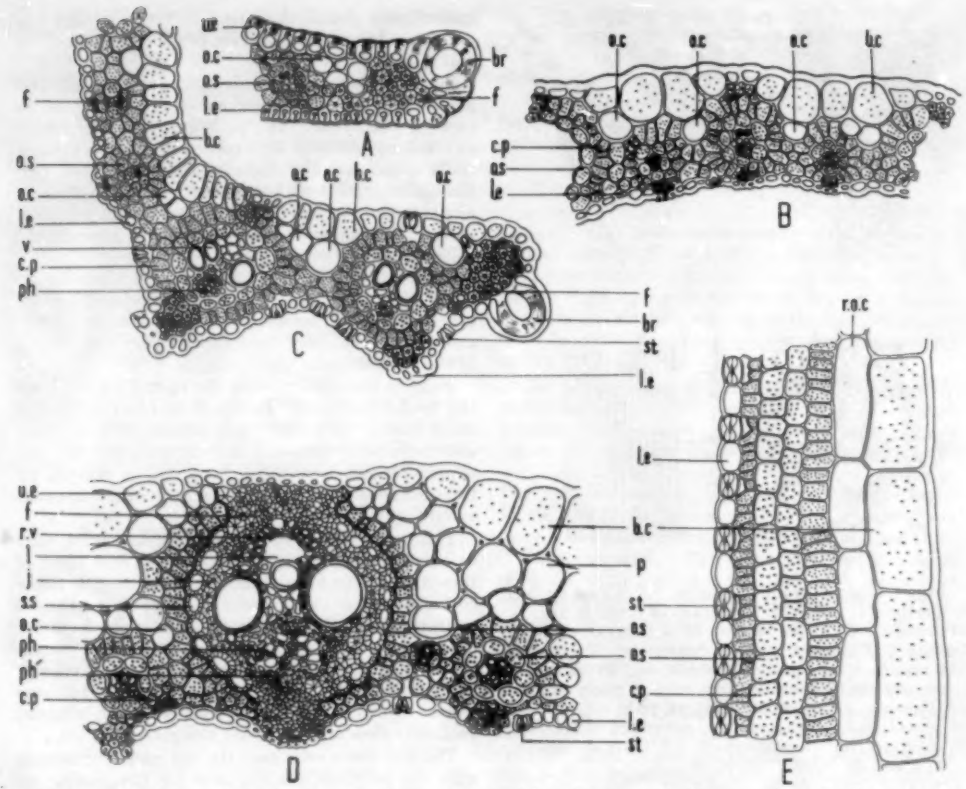

Fig. 5.—Structure of the blade. *A*. Cross section of basal part, including margin. *B*. Cross section midway its length. *C*. Cross section of upper part. *D*. Cross section midway its length, showing a large vein. *E*. Longitudinal section midway its length. *b.c*, bulliform epidermal cell; *br*, bristle; *c.p*, chlorophyll-bearing parenchyma; *f*, fibers; *j*, sclerenchyma jacket; *l*, protoxylem lacuna; *l.e*, lower epidermis; *o.c*, oil cell; *o.s*, outer sheath; *p*, parenchyma; *ph*, phloem; *ph'*, protophloem; *r.o.c*, row of oil cells; *r.v*, remains of vessel; *s.s*, sclerenchyma sheath; *st*, stoma; *u.e*, upper epidermis; *v*, vessel.
All, ×210.

98 to 160 oil cells averaging 24.5 microns in diameter. In the lower part of the blade were 41 to 96 such cells of a similar size in each section. In the upper part were 94 to 120 slightly smaller oil cells. In length the oil cells ranged between 56 and 147 microns.

The oil cells of the sheath (Figs. 6 and 7) are embedded in the central part of the fundamental parenchyma and form rows parallel to the long axis of the sheath. These oil cells are similar to those of the blade but are larger, averaging 65 microns in diameter and ranging from 88 to 455 microns in length. The oil cells may be of the same diameter as the surrounding parenchyma cells, but are always longer. A cross section of the sheath shows 43 to 98 oil cells, arranged singly or in small groups of two to five cells. They either are filled with a yellowish volatile oil or show it in small droplets. Protoplasmic remains can be detected in some of the oil cells.

The oil cells of the joint triangle are smaller in size and are embedded singly or in small groups in the parenchyma, usually closer to the outer side. Oil cells are absent in the tissues of the auricle, ligule, and culm.

General Features of the Epidermis.—The epidermis of the leaf (Fig. 8) has a structure like that of the grasses in general, and shows a design of alternating bands related to the tissues underlying it. The epidermis includes, among others, three related types of cells: (1) the long cells, (2) the suberized short cells, and (3) the silica cells. In addition there are the bulliform cells of the upper epidermis. Hairs and other emergences as well as typical monocotyledonous stomata are of frequent occurrence in the epidermal layers.

The long cells are rectangular although their ends are sometimes pointed or concave. In surface preparations stained with chlorozinciodide, the walls of the long cells acquire a bright blue color and have an undulate strongly silicified middle lamella which appears as a white wavy line. The outer periclinal walls are thick, silicified and suberized, and possess a more or less strongly developed cuticle. The inner and lateral walls have pits, which are wanting in the outer walls. In young tissue some of the long cells show peculiar circular pits in the anticlinal walls. Pee-Laby (4) stated that such pits affect the development of the undulations of the walls. These

circular pits remain unchanged during the growth of the walls and the sinuosity becomes more and more conspicuous while the organ is developing.

The long cells vary in size, measuring in the intercostal region of the blade up to 63 μ long, 17 μ wide, and 14 μ high. Over the veins the long cells measure up to 224 μ long, 11 μ wide, and 11μ high. In the sheath the dimensions of these cells are of the same order.

The short cells typically occur in pairs between the long cells. One member of the pair stains a golden yellow color with chlorozinciodide and is the suberized short cell. The other member appears glistening white and is the silica cell. Often, instead of two short cells constituting a group, a larger number is found; contrariwise, sometimes only a single suberized short cell is present between two long cells.

The suberized short cells have relatively thin refractive walls which are also somewhat silicified. They take a red stain when treated with Sudan III reagent. The cell cavities are large and usually contain a deposit of some organic substance. The cells usually are rectangular, especially when lying over the veins; however, they are sometimes broadly reniform or even triangular. The suberized short cell of a pair always lies at the apical end of the silica cell.

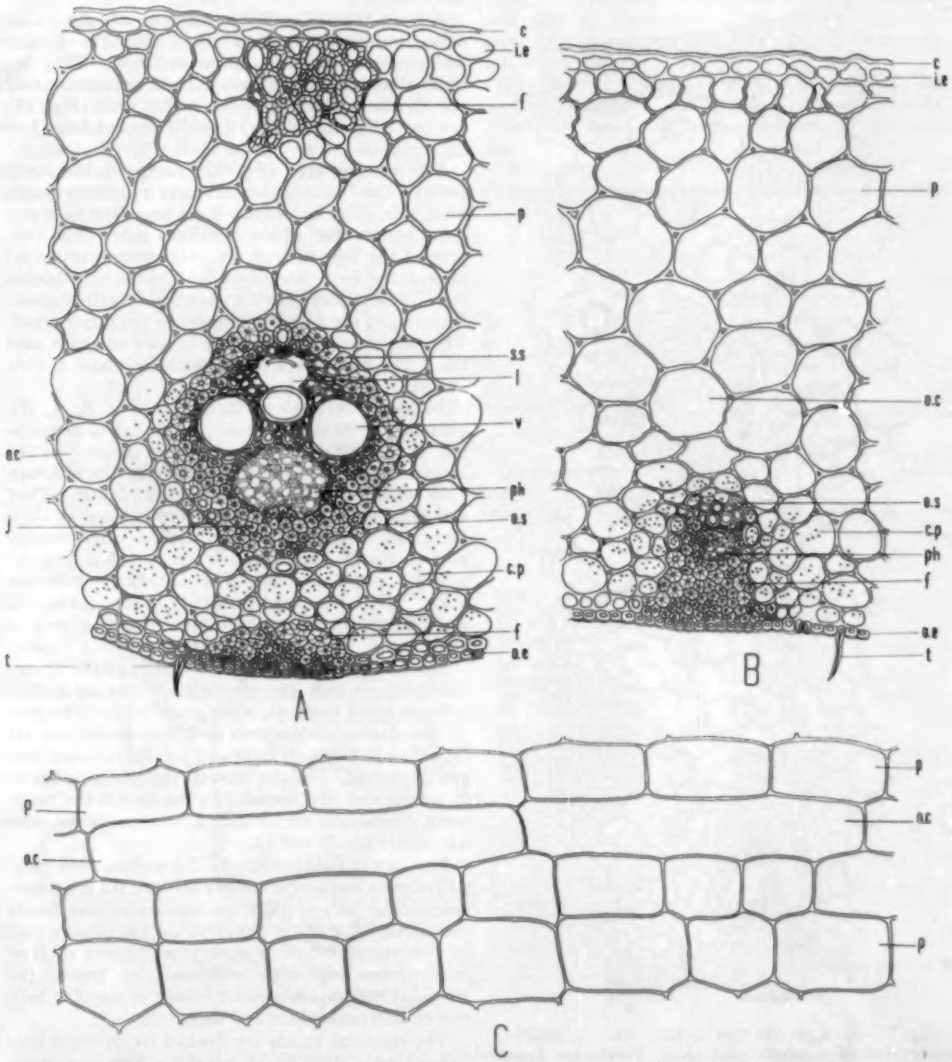

Fig. 6.—Structure of the sheath. *A*. Middle part with a large vein. *B*. Middle part with a medium large vein. *C*. Longitudinal section with oil cells. *c*, cuticle; *c.p*, chlorophyll-bearing parenchyma; *f*, fibers; *i.e*, inner epidermis; *j*, sclerenchyma jacket; *l*, protoxylem lacuna; *o.c*, oil cell; *o.e*, outer epidermis; *o.s*, outer sheath; *p*, parenchyma; *ph*, phloem; *s.s*, sclerenchyma sheath; *t*, trichome; *v*, vessel.
All, ×180.

The suberized short cells in the intercostal region measure up to 11 μ long, 14 μ wide, and 11 μ high. Over the veins they are up to 17 μ long, 11 μ wide, and 11 μ high. In the sheath they are not as high.

The silica cells are more uniform. They are usually rectangular and parallel to the leaf axis; occasionally they are oval or rounded, or even broader than long. In surface view they usually appear somewhat constricted in the middle. Silica cells do not occur in the lower epidermis of the blade.

Several types of trichomes occur, all nonglandular. The first (Figs. 8 and 9*B*) consists of the two-celled, appressed uniseriate hairs. Each is more or less cylindrical, with a small circular base and a blunt tip. It has a thin wall and a wide lumen. Of the two cells constituting the hair the basal one is usually somewhat longer. These are commonly found among the cells bordering the bands of stomata and sometimes are scattered among the stomatal rows. They usually occur in the blade and the sheath.

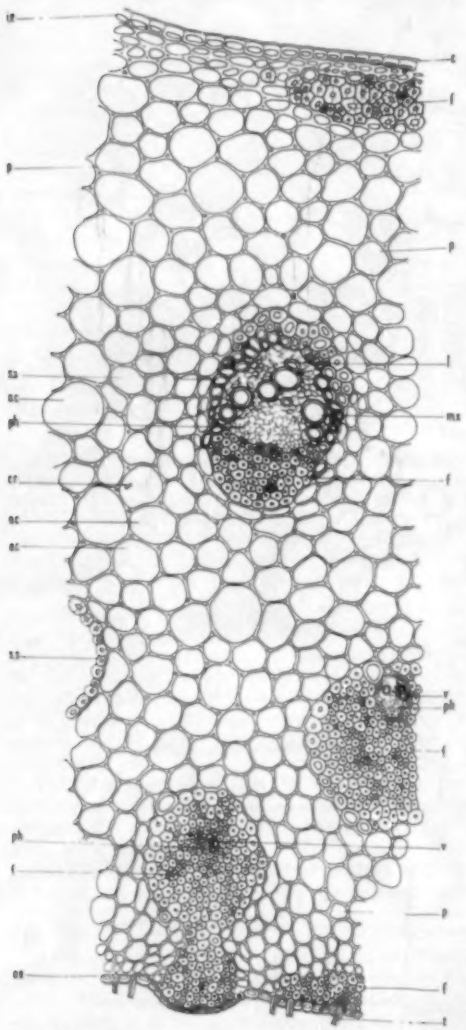

Fig. 7.—Structure of the sheath base. *c*, cuticle; *cr*, crystal of calcium oxalate; *f*, fibers; *i.e*, inner epidermis; *l*, protoxylem lacuna; *m.x*, metaxylem; *o.c*, oil cell; *o.e*, outer epidermis; *p*, parenchyma; *ph*, phloem; *s.s*, sclerenchyma sheath; *t*, trichome; *v*, metaxylem vessel. ×125.

The second type (Figs. 8 and 9*B*) consists of usually short, unicellular, thick-walled hairs with pointed tips. These vary in length, and may have the form of spines or bristles, especially over the veins and along the margins. These hairs and spines are usually pointed toward the upper end of the leaf.

A third type (Fig. 9*C*) is made up of long, cylindrical, unicellular lignified hairs. These have thick walls, narrow lumens, and pointed tips. They are quite abundant on the triangles and predominate on the sheath. Many of them on the ligule (Fig. 1*F*) are partially adnate to the epidermis but have free tips.

Still another type (Fig. 8*E*) occurs in the lower part of the blade and consists of relatively large, long, soft, silky unicellular hairs scattered here and there in the zone of the bulliform cells. The cells around the base of each hair are concentrically arranged and have thicker walls than the neighboring cells. These hairs have an average length of about 5 mm. and are usually straight or slightly curved. They are cylindrical, tapering toward an acute solid tip. The hair has a fairly thick wall and a wide lumen.

Bulliform cells, in surface view (Fig. 8, *A*, *E*), are more or less quadrangular, hexagonal, or pentagonal, or sometimes almost square in shape. In the cross section (Fig. 5) they differ from the ordinary epidermal cells chiefly in their greater depth. They are usually narrower at their outer than at their inner end, where they touch the chlorophyll-bearing parenchyma. They measure up to 115 μ long, 59 μ wide, and 87μ high. The anticlinal walls are fairly thin and slightly wavy. Both tangential walls of these cells are thin and usually convex in curvature. The outer walls are covered by cuticle.

Stomata (Figs. 4*D*, 8, and 9) are typically monocotyledonous and phaneroporous. The subsidiary cells are much larger than the guard cells. The pore of the stoma is elongated and lozenge-shaped, its lateral walls being straight and parallel or sometimes gently curved. In the lamina the stoma is up to 21 μ long and 10 μ broad. In the sheath the maximum dimensions are 32 × 21 μ, whereas in the culm the values are 28 and 11.

The Lower Epidermis.—In the surface view (Fig. 8*B*) of the lower epidermis of the blade the most conspicuous groups of cells are the intercostal bands containing stomata. These strips are usually two to five rows wide and each row consists of long cells alternating with stomata. At times, the stomatal rows are separated from one another by a row of alternating long and short cells.

The stomatal bands are flanked by strips of long narrow cells that lie above the sclerenchymatous bands. The narrowest strips are made up of a few rows containing long cells alternating with suberized short cells. The middle row of a broader band, and sometimes two adjacent rows, is made up en-

tirely or partly of suberized short cells. Silica cells are absent.

In cross sections these lower epidermal cells are narrower and shorter than those in the intercostal zones. They have thicker, nonlignified pitted walls.

The Upper Epidermis.—Except for the added presence of bulliform cells and silica cells, the cells making up the upper epidermis of the leaf blade (Fig. 8, *A*, *C*) are the same as those found in the lower epidermis though in different proportions. The bulliform cells are easily the most outstanding feature of the upper epidermis. Depending on the relative size of the veins and their spacing, the bands of bulliform cell rows are wide or narrow, crowded or far apart.

The tissue between two bands of bulliform cells lies over a band of sclerenchyma of the large or medium large bundles. It is composed of a few rows of long cells alternating with suberized short cells and silica cells. Rarely, short spines are found arising in the middle row, which may be made up partly of short cells.

At each side of a band lying over a vein, there is a marginal zone one to five cells wide formed of long cells alternating with silica or suberized short cells, single or in pairs. These have more undulate walls and are broader, especially toward the band of bulliform cells. The cells nearest the bulliform cells usually show projections from the wall into the cell cavity. Occasionally bicellular hairs of the type found in the lower epidermis occur in this zone. Stomata may be present in a continuous or interrupted row in the marginal zone, especially along the large veins.

Other Epidermis.—The outer epidermis of the sheath (Fig. 9) differs from the corresponding part of the lamina in having fewer stomata. The intercostal band consists of regular long cells alternating with one or two silica or suberized short cells. Some of the short cells are replaced by trichomes. The epidermis overlying a vein consists of narrower cells and has no trichomes. This area is separated from an intercostal band by a single row of stomata and long cells.

The inner epidermis of the sheath consists of large and uniformly rectangular cells with no short cells. Trichomes are absent but a few stomata are present.

The epidermal layers of the auricles are continuous with those of the sheath and appear similar.

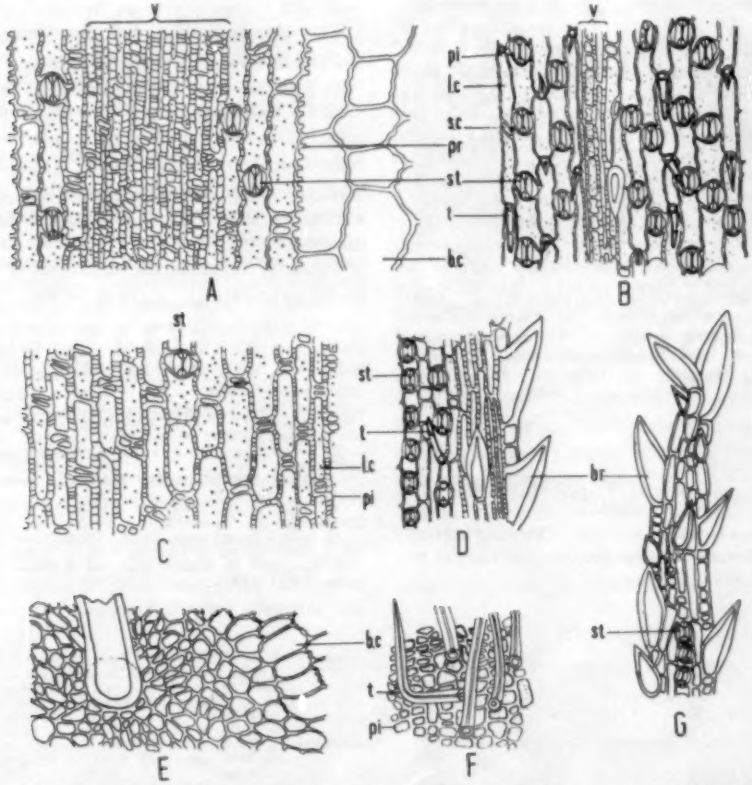

Fig. 8.—Epidermis of the blade. *A*. Upper epidermis from middle part. *B*. Lower epidermis from middle part. *C*. Upper epidermis from middle part in midrib region. *D*. Lower epidermis from upper part showing the margin. *E*. Upper epidermis from basal part showing base of a silky hair. *F*. Lower epidermis of blade joint. *G*. Tip of blade showing lower epidermis. *b.c*, bulliform cell; *br*, bristle; *l.c*, long cell; *pi*, pit; *pr*, projection; *s.c*, short suberized cell; *st*, stoma; *t*, trichome; *v*, vein region.
All, ×210.

TABLE I.—DISTRIBUTION OF ELEMENTS IN THE LEMONGRASS LEAF

	Blade	Sheath	Culm	Joint
Bundles in cross sections	In one row near lower epidermis	In several rows near the base	Scattered	None in ligule. Present in auricles
Suberized and long cells	In both surfaces	In outer epidermis only	Present	None in ligule or inner epidermis of auricles
Silica cells	In upper epidermis only	In outer epidermis only	Present	None in ligule or inner epidermis of auricles
Trichomes	On both surfaces	On outer epidermis only	None	On outer epidermis only of ligule and auricles
Stomata	Fewer in upper epidermis	Fewer than in blade	Present	None in ligule. Present in auricles
Oil cells	Directly under the bulliform cells	In middle of parenchyma	None	None in ligule or auricles
Number of oil cells in a central x-section	98–160	43–98	None	None

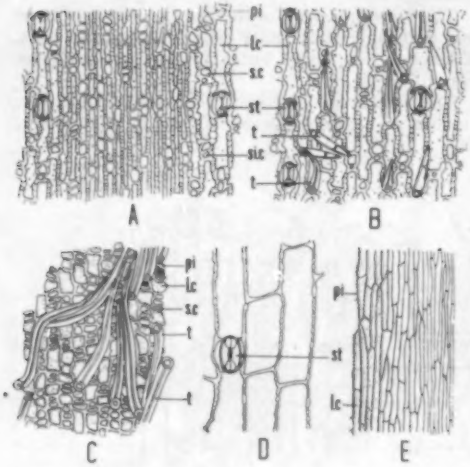

Fig. 9.—Epidermis of the sheath. *A*. Outer epidermis over a large vein. *B*. Outer epidermis over an intercostal region. *C*. Outer epidermis of sheath base. *D*. Inner epidermis. *E*. Outer epidermis at margin. *l.c*, long cell; *pi*, pit; *si.c*, silica cell; *s.c*, suberized short cell; *st*, stoma; *t*, trichome.
All, ×170.

The epidermal cells of the ligule (Fig. 1*F*) are rectangular in surface view. Stomata are absent. The outer epidermis is covered with long partially adnate trichomes, whereas the inner or adaxial surface is glabrous.

The epidermis of the culm (Fig. 4*D*) is remarkably uniform in appearance, being made up of narrowed long and short cells whose walls are straighter than elsewhere in the leaf. The stomata are narrower also.

SUMMARY

The structure of the lemongrass leaf in its various parts is described. The volatile oil occurs internally in rows of parenchymatous cells, slightly but distinctly different from adjacent cells. The lower part of the blade has fewer oil cells than the remainder of the leaf. The sheath also has fewer oil cells but they are larger, averaging 65 microns in diameter as against 24.5 microns.

Table I compares the distribution of certain elements in different parts of the leaf.

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2-Nitro-2-methylpropyl Carbamate*

By DONALD KROOK and C. E. MILLER†

2-Nitro-2-methylpropyl carbamate has been prepared by the reaction between 2-nitro-2-methylpropanol-1 and liquid phosgene followed by amination with concentrated ammonium hydroxide. This new carbamate has been characterized by the formation of certain derivatives.

THE DRUG URETHAN (1) has been used in the treatment of human leukemia and has inhibited the growth of some animal tumors. A large number of urethans have been prepared and tested (2, 3). A survey of the literature, however, failed to disclose the existence of any nitroalkyl carbamates.

Our interest in the introduction of a nitro group in the urethan nucleus centered about a project under way in which new derivatives of the drug urethan and its homolog are being prepared for screening as antileukemic agents. The purpose of this paper is to present our method of preparation of the hitherto unknown 2-nitro-2-methylpropyl carbamate. A typical run is described.

EXPERIMENTAL

Carboalkoxylation.—The reactor used in this work was a 1,000-cc. three-necked flask fitted with a jacketed gas inlet tube, mechanical stirrer, and a reflux condenser. The flask was immersed in an ice bath and 63 Gm. (0.63 mole) of liquid phosgene was transferred to the reactor from a cylinder of phosgene. The phosgene was diluted with 100 cc. of ether, and 69 Gm. (0.58 mole) of 2-nitro-2-methylpropanol-1 dissolved in 100 cc. of ether was added to the phosgene solution over a period of one hour. Stirring was continued for six hours and the reaction mixture was allowed to warm to room temperature and left overnight.

Amination.—The ethereal solution of 2-nitro-2-methylpropyloxy-carbonyl chloride was added slowly with stirring to an excess of concentrated ammonium hydroxide while maintaining the temperature at from 5 to 10° by an ice bath. After all of the chloroformate had been added to the ammonia solution a stream of gaseous ammonia was bubbled through the reaction mixture until an excess of ammonia was present. The ethereal solution was separated in the usual manner and upon evaporation gave 58 Gm. (62% yield) of colorless crystals, insoluble in water, soluble in chloroform and ether. A sample recrystallized from chloroform for analytical use melted at 77–79°.

Anal.—Calcd. for $C_5H_{10}N_2O_4$: C, 37.03; H, 6.15; N, 17.15. Found: C, 37.04; H, 6.19; N, 17.12 (Dumas).

The 2-nitro-2-methylpropyl carbamate was further characterized by the preparation of certain derivatives following the method of Sawicki (4).

Di-(2-nitro-2-methylpropyl)-propylidenedicarbamate.—A drop of concentrated hydrochloric acid was added to a solution of 1.62 Gm. of 2-nitro-2-methylpropyl carbamate in 2 cc. of dimethylformamide and 1.00 Gm. of propionaldehyde. The mixture solidified upon standing. Recrystallization from dioxane-water gave colorless crystals melting at 189–190°.

Anal.—Calcd. for $C_{13}H_{24}N_4O_8$: N, 15.38. Found: N, 15.45 (Dumas).

Di-(2-nitro-2-methylpropyl)-butylidenedicarbamate.—This compound was prepared by the same procedure used for the propionaldehyde derivative except that *n*-butyraldehyde was used. Recrystallization from dioxane-water gave colorless crystals, m. p. 155–157°.

Anal.—Calcd. for $C_{14}H_{26}N_4O_8$: N, 14.81. Found: N, 14.64 (Dumas).

LD_{50} Determination.—LD_{50} values for 2-nitro-2-methylpropyl carbamate were determined on week-old chicks. Administered subcutaneously in propylene glycol the LD_{50} was found to be 1,175 mg./Kg. with limits of error of 96–104% (5).

SUMMARY

1. The nitroalkyl carbamate, 2-nitro-2-methylpropyl carbamate, has been prepared by the reaction between the corresponding chloroformate and ammonium hydroxide.

2. Several alkyl derivatives of the nitrocarbamate have been prepared.

3. The LD_{50} values of the nitroalkyl carbamate have been determined.

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* Received August 2, 1954, from the School of Pharmacy, North Dakota Agricultural College, Fargo.
Acknowledgment is given to the North Dakota Cancer Society for financial assistance which made this work possible.
Presented to the Scientific Section A. PH. A. Boston meeting, August, 1954.
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Further Studies on Texas Plantago Seeds*

By MARTHA JANE JONES† and C. CLARENCE ALBERS‡

A comparative study was made of the seeds of the official *Plantago ovata* Forskal, the homegrown variety of *Plantago ovata*, and three Texas Plantago species: *Plantago Helleri* Small, *Plantago inflexa* Morris, and *Plantago rhodosperma* Decne. The investigation was carried out by making morphological and histological comparisons of the seeds and quantitative and qualitative comparisons of the mucilage content. It was found that the mucilage content of each of the Texas species is slightly less than that of *Plantago ovata* Forskal. However, the abundant occurrence and ease of cultivating and harvesting of the Texas species make them an excellent potential commercial crop. The homegrown variety of *P. ovata* was found to yield a percentage of mucilage equal to that of the imported variety, and commercial production of this species in the United States would seem to be warranted.

PSYLLIUM SEED MUCILAGE has attained considerable importance in the past few years, not only in the preparation of medicinal laxatives, but also in the arts and industries, such as the cosmetic and the textile industries. The mucilage for these purposes is obtained primarily from *Plantago ovata* Forskal (Blonde psyllium) and is imported from India chiefly as "Psyllium husks." The blond seeds are preferred as a source for this product, not only because they yield a practically colorless mucilage, but primarily because their mucilage layer cracks off under slight mechanical pressure applied to the seed and can readily be separated from the seed. One importer reports that his firm has not been wholly satisfied in its dealings with Indian sources, hence it has undertaken experimental cultivation of this species in the United States, especially in Arizona.

Greenberg (1) has directed attention to at least one Texas species of Plantago, namely *Plantago Wrightiana* Decne, as a possible source of mucilage and has made some quantitative and qualitative comparisons with the official seeds. The present work is a continuation of such studies applied to several additional native species of Plantago as well as to cultivated *P. ovata*. In addition to the study of the mucilage forming capacity of each species a morphological and histological study of the seeds was also made.

MATERIALS

The seeds designated in this study as *Plantago inflexa* Morris, *Plantago Helleri* Small, and *Plantago rhodosperma* Decne were obtained from plants authenticated by Dr. B. C. Tharp, Curator of the Herbarium, the University of Texas.

* Received April 12, 1954, from the University of Texas, College of Pharmacy, Austin.
Abstract of a thesis presented to the Graduate School of the University of Texas in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy, August, 1950.
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Plantago inflexa Morris.—The seeds of this species were collected in June, 1949, in Washington County, Texas. *P. inflexa* which is native to Texas was found in dry, deep sandy soil. The scapes of the plant were erect, very strong, and extended well beyond the leaves. The fruiting spikes, which varied in number per plant, were quite coarse and measured from 2 to 4 inches in length. The color of the seeds varied from light to dark brown.

Plantago Helleri Small.—The seeds of *P. Helleri* were obtained from plants collected in late June, 1950, north of Austin, Texas. This plant, smaller and less conspicuous than the other Texas species which were studied was usually only 5 inches high. This species is native to Texas and is found in dry, limestone soil. The spikes were oblong-cylindrical and measured up to $3^1/_2$ inches in length, usually extending only a short distance above the leaves. The seeds were medium brown in color.

Plantago rhodosperma Decne.—The seeds of this species were collected from plants grown in the Medicinal Plant Garden of the College of Pharmacy, the University of Texas, during 1948 and 1949. *P. rhodosperma* is found in sandy soil from Missouri and Oklahoma to Louisiana, Texas, and Arizona (2). In the Austin area it attains its maximum development in heavy limestone soils. The scapes were erect, usually extending above the leaves. The spikes measured from 2 to $7^3/_4$ inches in length and were densely flowered. The capsules dehisce less readily than those of the other species, making it more difficult to remove the dark red seeds. This would be an advantage in the harvesting of the crop since there would be less shattering and loss of the seeds.

Many of the plants collected near Austin were very large in comparison with the other Texas Plantagos. One plant was found to be 13 inches high and to have 63 fruiting spikes. The length of the spikes ranged from $3^1/_2$ to $7^3/_4$ inches, and the number of seeds from each spike varied from 190 to 218 and weighed from 108 to 208 mg. The total weight of the seeds from the entire plant was 9.978 Gm.

Plantago ovata (homegrown).—The cultivated *Plantago ovata* seeds were collected from plants grown in the Medicinal Plant Garden of the College of Pharmacy from seeds supplied by the Serutan Company. The seeds were planted in beds in sandy loam soil in the late fall of 1948 and the crop harvested in May, 1949. The scapes varied from one to several per plant, rarely surpassing the leaves.

The spikes of these cultivated plants measured up to 1 inch in length and produced three types of seeds: silvery gray, partly gray and partly light brown, and entirely light brown.

Harvesting of all seeds was done by hand and the cleaning process was accomplished by winnowing with an electric fan.

EXPERIMENTAL

Swelling Factors.—In earlier studies Burlage (3) reported a wide variation in the swelling factor within the same lot of seeds of Plantago species. Because of such reports, methods were employed in order to determine if the official N. F. (4) method (I) and the procedure used by Greenberg (1) (II) had any effect on the swelling factor. Results obtained by each method are recorded in Table I.

Mucilage Content.—The seeds used in the swelling tests according to Method No. I were utilized as a source of the mucilage for the quantitative determinations. The mucilage was separated from the seeds by use of the hand press devised and described by Greenberg (1). The seeds were previously dried at 98° for ten hours before weighing. At the end of the twenty-four-hour period, as described in Method No. I, the contents of the cylinder were transferred to the hand press and the expressed mucilage collected in a tared evaporating dish. Both the cylinder and the hand press were washed two or three times, and the wash water added to the mucilage. The seeds were then carefully transferred to another tared evaporating dish. The evaporating dishes and their contents were thoroughly dried at 98° to a constant weight and the mucilage percentage was determined by (*a*) direct weight of the dried expressed mucilage, and (*b*) indirectly by loss of weight of the seed during the operation. The results are tabulated in Table I.

It will be noted that the figures check rather closely for all species except *P. rhodosperma* and show that very little mucilage is lost in the hand press. The discrepancy in the case of *P. rhodosperma* is apparently due to a greater tenacity of the mucilage of this species which interferes with its complete removal from the cylinder and hand press during the operation.

The mucilage separated from the seeds of commercial *P. ovata*, homegrown *P. ovata*, and *P. rhodosperma* was a clear jellylike mass varying in color with the different species. Mucilage expressed from *P. Helleri* and *P. inflexa* seeds was a dirty-white, translucent mass. When examined closely, denser areas occur thickly scattered throughout the gelatinous mass. Greenberg (1) has referred to these dense areas as incompletely hydrated mucilage forcibly expressed from the seed.

The powdered mucilage from the different species possessed the following colors: pale cream color, *P. Helleri* and *P. inflexa;* brownish-tan, *P. rhodosperma;* grayish brown, *P. ovata* (homegrown); light tan, rose tint, *P. ovata* (commercial).

The solutions of the various mucilages varied in color and in viscosity. The latter property is tabulated in Table II.

Viscosity Determinations.—The dilutions used in determining the viscosities were prepared by adding 150 mg. of the powdered mucilage, dried at 98° to constant weight, to 20 cc. of distilled water. The determinations were made at 37.8° using the Ubbelohde viscosimeter and the results reported in Table II.

For the first trial the powdered mucilage was added to the water in a 125-cc. Erlenmeyer flask and allowed to stand, with occasional shaking, for eight hours. At the end of this period only the mucilage from the homegrown *P. ovata* yielded a clear, transparent liquid. The viscosity of this solution was found to be 19.02. The mucilage of the remaining species required a period of heating, ranging from fifteen to twenty-five hours, to effect a homogeneous solution for the determinations. The heating process was carried out by loosely corking the flasks and placing in an oven at 98°. After cooling to

TABLE I.—SWELLING FACTORS, MUCILAGE CONTENT, AND SIZE OF SEED

Species	Swelling Factors[a] (Av. Volume), cc. Method I	Method II	Av. Percentage of Mucilage[b] Direct	Indirect	Av. Wt. of 100 Seeds,[c] Mg.	Size of Seeds in mm.[d] Length	Width
P. inflexa	12.5	13.5	17.7	18.3	184.01	2.5–3.0	1.5–1.75
P. Helleri	16.5	16.0	17.4	17.7	229.93	3.25–3.5	1.5–1.75
P. rhodosperma	18.5	18.0	17.5	21.0	151.81	2.5–3.24	1.25–1.5
P. ovata (homegrown)	10.5	11.0	21.8	22.1	148.48	2.5–3.0	1.5–1.75
P. ovata (commercial)	11.5	10.0	21.5	21.7	189.71	3.0–3.25	1.5–1.75

[a] At least 5 determinations were made on each species.
[b] At least 5 determinations were made on each species.
[c] Six determinations were made on selected seeds of each species.
[d] Ten determinations were made on selected seeds of each species.

TABLE II.—KINEMATIC VISCOSITIES (CENTISTOKES)

Species	First Trial (Allowed to Stand for 8 Hours Then Heating from 0–25 Hours)	Second Trial (Heating for 34 Hours)	Third Trial (Allowed to Stand in Refrigerator for 12-Hours—Heating for 74 Hours) 5 Min.	12 Hrs.	18 Hrs.	32 Hrs.	74 Hrs.
P. inflexa	9.80	11.08	7.11	5.76	5.76	5.76	3.87
P. Helleri	6.63	4.96	17.39	7.94	6.84	5.53	3.54
P. rhodosperma	2.89	4.33	Too thick	Too thick	Too thick	1,445.00	2.93
P. ovata (homegrown)	19.02	3.85	177.38	45.01	26.83	17.83	3.31
P. ovata (commercial)	21.19	4.76	224.38	36.32	21.92	21.92	2.75

37.8° the mucilage was introduced into the viscosimeter and five determinations were made, in rapid succession, and the average was taken as the viscosity value.

Inasmuch as the solutions prepared by the above method were not uniformly treated, the values obtained were not truly comparable; therefore, a second trial was carried out with the solution of each mucilage subjected to similar treatment. The powdered mucilage was added to the water in a 50-cc. cylinder. The cylinder was then vigorously shaken and immediately placed in the oven and heated for thirty-four hours. During this treatment water lost by evaporation was replaced and the solutions were cooled to 37.8°.

Since the results obtained from trials 1 and 2 varied considerably and because it was observed that heating over a prolonged period of time obviously had some effects on the mucilages, a third trial was attempted to investigate further the causes for these vast differences in the viscosity values. Each mucilage was accordingly prepared by adding the powdered mucilage to the water in a test tube, placing it in the refrigerator, allowing it to stand for twelve hours, then transferring the test tube to a large beaker of boiling water and heating for five minutes. Each test tube was thereupon shaken vigorously to dissolve any small clumps of undissolved mucilage, after which the solutions were then placed in the oven and heated at 98° for a total of seventy-four hours, and the viscosity determinations were made at intervals of twelve-, eighteen-, thirty-two-, and seventy-four-hour periods of time, respectively. The results reported show a steady decrease in the kinematic viscosity of the solutions.

In analyzing the viscosities reported in Table II, second trial, it will be observed that after heating for thirty-four hours all the mucilages, except that from *P. inflexa*, show approximately the same values. Furthermore, it will be noted that the viscosities reported in the first trial, where the solutions were allowed to stand before heating, were higher than those obtained in the second trial, with the exception of those of *P. inflexa* and *P. rhodosperma* which were shown to be somewhat lower in the first trial. From these results it might be assumed that allowing the mucilage to stand for eight hours before heating tends to facilitate, in most instances, the hydration of the mucilages, causing an increase in the viscosities. If the mucilages are heated for thirty-four hours immediately after they are placed in water, heating either prevents hydration or else causes some other physical or chemical change in the colloidal solution, after hydration takes place, to cause a decrease in the viscosity. The mucilages from *P. inflexa* and *P. rhodosperma*, which have been the exceptions to the general trend in the previous trials, also show a difference in the third trial in that they change very little over a thirty-two-hour heating period, whereas the other mucilages steadily decrease after each period of heating. The behavior of *P. rhodosperma* mucilage indicates that it does not hydrate rapidly (requiring twelve hours to form a very thick mucilage); however, once it is hydrated it remains stable toward heat for a period of thirty-two hours before undergoing further change, either through hydrolysis or in the colloidal state of the solution. After thirty-two hours the solution is still extremely viscous, but measurable, but upon further heating the change in viscosity occurs quite rapidly to attain the value of 2.93 at the end of the seventy-four-hour period. Although the various mucilage solutions vary considerably under different treatment, it will be noted that after heating for seventy-four hours all the mucilage solutions tend to approach the same viscosity value.

The conclusions drawn from the viscosity values reported are that (*a*) heating has a definite effect on the mucilage solutions, (*b*) the period of time each solution is allowed to stand before heating is a significant factor, and (*c*) all the mucilages tend to approach the same viscosity value if heated for a long period of time.

A definite statement as to what changes occur in the mucilages to cause these variations in the viscosities cannot be made. A question arises as to the possibility of the change being brought about by enzymatic action, but since enzymes are thermolabile at 98° some other explanation must be sought. Instead, it appears to be a physical or chemical change brought about by hydrolysis. Hydrolysis might occur before, during, or after the process of hydration; or it is possible that a change in the micellar structure of the mucilage solution occurs. More information concerning the physical and chemical properties of the mucilages will have to be obtained before a satisfactory explanation can be given.

Since the viscosities vary so markedly under different conditions of time and temperature, it would be difficult to correlate this property with the medicinal and industrial use of the mucilages.

Microtechnique Methods.—In previous histological studies on certain Plantago seeds, Neva (5) used, with special adaptations, the double embedding procedure recommended by Johansen (6) and Sass (7) in getting suitable sections for study. It was found in the present study that if the fresh mature fruits were used, only the paraffin method was necessary to obtain satisfactory transverse sections of the seeds. Following Neva's (5) general procedure, with some modifications, complete cross sections of the seeds with the mucilage layer still intact were obtained.

The seeds were placed for five days in a formalin-aceto-alcohol solution in which the water concentration was approximately 16%. They were then washed with 95% alcohol and absolute alcohol, respectively. After washing, the seeds were then hardened and dehydrated by passing them, in succession, through 10, 30, and 60% chloroform-absolute alcohol mixtures and finally into 100% chloroform. After hardening with chloroform the paraffin method described by Sass (7) was employed. Sectioning was accomplished by (*a*) using a rotary microtome and (*b*) making freehand sections. The sections were stained by passing them, in succession, through 95% alcohol, the alcoholic staining solutions of 0.5% safranin and 0.5% fast green, absolute alcohol, and finally into xylol.

DESCRIPTION OF THE SEEDS

External Morphology.—A binocular dissection microscope (17×) was used in the examination of the external morphology of the seeds.

Plantago inflexa.—The seeds of *P. inflexa* measured from 2.5 to 3.0 mm. in length and from 1.5 to

1.75 mm. in width. They were ovate-oblong, typically boat-shaped, light brown over most of the top portion of the seed but darker brown along the seed margin. The light brown area visible on the convex dorsal surface coincided with the location of the straight embryo or possibly outlined the cavity on the concave ventral surface. This area extended almost the entire length of the seed and the division between the two cotyledons could easily be seen.

The dorsal surface was dull, obscurely papillose, and exhibited a distinct transverse groove near the mid-section of the seed.

The concave ventral surface showed a narrowed cavity bordered by a dark brown somewhat flattened ridge. The ventral hollow was completely covered with a white scaly tissue, leaving only the hilum exposed.

Plantago rhodosperma.—The seeds of *P. rhodosperma* varied in length from 2.5 to 3.24 mm. and in width from 1.25 to 1.5 mm. They were broadly ellipsoidal, pear-shaped, and light to dark red in color.

The convex dorsal surface exhibited a light-colored elongated central area, tapering at the broader end of the seed. The margin of the seed appeared as a white translucent band, especially conspicuous at the two extremities.

The ventral surface was slightly concave bordered by a clear, thin, low ridge. Near the center of the ventral surface lay the distinct white hilum. Both the dorsal and ventral surfaces were dull and slightly papillose.

Plantago Helleri.—The seeds of *P. Helleri* were larger than those of the other two Texas species, varying in length from 3.25 to 3.5 mm. and in width from 1.5 to 1.75 mm. They were ovate-ellipsoidal, larger at one end than the other, medium brown, but darker brown along the seed margin.

The convex dorsal surface was dull, slightly papillose, and showed a transverse groove nearer the broader end.

The concave ventral surface, showing a large cavity with the hilum located near the center of the base of this cavity, was bordered by a dark-brown flattened ridge. Most of the furrowed surface was covered with a white scaly tissue which was more dense around the hilum and around the base of the ridge.

Plantago ovata (homegrown).—The homegrown *P. ovata* produced seeds which varied considerably in appearance. There were three types: The first type was like the usual commercial form with the silvery-white layer covering all but a small central area on the dorsal side of the seed. They differed only in that they were slightly smaller. The second type was only partly covered with the silvery-white coating. In some instances one-fourth or one-fifth of the seed was covered with the white layer, the remaining portion being a light-brown color. The dorsal surface of some of the seeds showed only a narrow strip of the white coating formed across one end or along one side of the seed. The silvery-white coating seemed to be deposited in a very irregular fashion without any definite pattern. The third type possessed no silvery-white coating, but otherwise resembled the official variety. This last type of homegrown *P. ovata* seed was light brown in color. The dorsal surface showed a yellowish-brown center extending almost the full length of the seed, fading into a light brown (chestnut brown) border.

Fig. 1.—Transverse section of *Plantago inflexa* Morris seed showing cotyledons, endosperm, and outer mucilage layer. × 50.

The homegrown *P. ovata* seeds measured 2.5 mm. in length, rarely up to 3.0 mm., and from 1.5 to 1.75 mm. in width.

The silvery-white coating of these seeds appeared to be an extra thick layer of mucilage. Since the majority of the seeds obtained from the homegrown variety possessed no white coating, it was assumed that they would yield a smaller per cent of mucilage than the official variety. Experimental results, however, showed this assumption to be in error, as the quantitative yield of mucilage was approximately equal to that of the official variety, as shown in Table I.

Histology.—Transverse sections of the seeds of *P. inflexa* (Fig. 1), *P. rhodosperma* (Fig. 2), *P. Helleri*, and *P. ovata* (homegrown) were made and the three distinct regions, the embryo, endosperm, and seed coat were examined under low- and high-power magnifications.

Plantago inflexa.—*Embryo.*—The straight embryo was located in the center of the endosperm and consisted of two heel-shaped cotyledons adhering in the direction perpendicular to the ventral surface. Each cotyledon was composed of compact, circular, thin-walled parenchyma cells with the outermost layer being formed by slightly smaller, nearly square cells.

Endosperm.—The endosperm extended from both sides of the embryo in a curved ventral direction to form the two ridges which outlined the ventral cavity. These ridges conspicuously folded toward the center of the seed. The endosperm was composed of thick-walled, irregular shaped cells, with the

Fig. 2.—Transverse section of *Plantago rhodosperma* Decne seed showing cotyledons, endosperm, pigment layer, and outer mucilage layer. × 50.

outermost layer consisting of thick-walled palisade cells measuring from 20–28 μ in height.

Seed Coat.—The seed coat consisted of (*a*) the pigment layer, and (*b*) the outer epidermal layer. The pigment layer surrounded the endosperm and was formed by a single layer of flattened, wavy, somewhat collapsed, thin-walled cells measuring up to 8 μ in height. The single layer of outer epidermal cells was thin-walled, translucent, mucilaginous cells varying from a rectangular to a nearly square shape. On most of the dorsal surface, the cells measured in height from 30–40 μ. Along the incurved margins of the seed, the mucilaginous cells became somewhat elongated, measuring from 90–110 μ in height. At the top of the endosperm ridge, the region where the endosperm folded back toward the center of the seed, the outer epidermal layer became detached from the pigment layer and passed directly to the ventral surface near the hilum.

Plantago rhodosperma.—*Embryo.*—The two cotyledons of the embryo adhering in the direction parallel to the ventral surface, appeared as somewhat flattened, elongated semicircular structures. Each cotyledon consisted of several layers of compact, thick-walled, rectangular cells with ends being somewhat pointed, presenting a "storied cambial" effect.

Endosperm.—The endosperm did not form two ridges on the ventral surface as distinctly as those observed in other Plantago species. It formed a somewhat straightened half-moon shape, with the ends slightly rounded, the center region on the concave ventral surface being slightly raised. The cells, however, which made up the endosperm, were very similar in structure to those of *P. inflexa*, the palisade cells measuring from 8–15 μ in height.

Seed Coat.—The pigment layer differed considerably from that of *P. inflexa*. It was composed of rectangular and square shaped cells measuring from 12–28 μ in height and from 8–15 μ in width. The cells contained a dark red pigment. The single outer epidermal layer strongly adhered to the pigment layer around the entire seed, leaving only the hilum exposed. The clear, translucent cells were rectangular in shape measuring from 20–40 μ on most of the dorsal surface, 15–20 μ on the ventral surface, and from 70–90 μ near the ends of the half-moon shaped endosperm.

Plantago Helleri.—*Embryo.*—The two cotyledons of the embryo of *P. Helleri* were very similar to those of *P. inflexa*. They differed from *P. Helleri* in shape in that they were slightly larger and more rounded in appearance.

Endosperm.—The endosperm of *P. Helleri* differed from that of *P. inflexa* in that the endosperm ridges formed are thinner and fold toward the center of the seed to a lesser extent than those of *P. inflexa*. The cells of the palisade layer measured from 10–15 μ in height.

Seed Coat.—The cells making up the single pigment layer were structurally similar to those found in *P. inflexa*. They contained a dark brown pigment and measured from 5–8 μ in height. The cells which form the outer epidermis resembled those described under *P. inflexa*. They varied in height from 30–40 μ on most of the dorsal surface, to 90–110 μ along the incurved margins of the seed. The epidermal layer also became detached from the pigment layer near the top of the endosperm ridges; it did not extend directly to the ventral surface, but followed closely the cavity outline.

Plantago ovata (homegrown).—*Type I.*—The histology of this type seed produced by homegrown *P. ovata* was identical to that of *P. ovata* commercial, the description of which has been adequately covered in previous works. It was observed in this study, however, that the outer epidermal layer adhered to the pigment layer along the central area of the dorsal surface and along the top portion of the ventral endosperm ridges. It was detached from the pigment layer on the remaining portion of the seed. This could be the explanation for the particular coloration of the seed. The intercellular area between the pigment layer and the epidermal layer would tend to obscure the brown pigment layer, thus giving the seed a whitish appearance.

Type III.—The histology of the brown *P. ovata*, homegrown, also resembled that of the official variety. It differed from the latter in that the mucilage layer closely adhered to the pigment layer, which completely enclosed the seed.

SUMMARY

A study has been made of three Texas Plantago species and of the homegrown *P. ovata*. The morphological and histological characteristics of the seeds of each of these species have been described, the mucilage content of each has been quantitatively determined, and a study of the effects of time and temperature on the viscosity of the various mucilage solutions has been made. From these studies the following observations were made:

1. The mucilage content of each of the three Texas Plantagos, *P. inflexa*, *P. rhodosperma*, and *P. Helleri* was slightly less than that of *P. ovata* (commercial).

2. The homegrown *P. ovata* produces three types of seed, the majority of which differs in appearance from the imported seed of *P. ovata*. It was found, however, that the homegrown variety yielded a percentage of mucilage equal to that of the official variety.

3. The conclusions drawn from the viscosity values reported are that (*a*) heating has a definite effect on the mucilage solutions, (*b*) the period of time each solution is allowed to stand before heating is a significant factor, and (*c*) all the mucilages tend to approach the same viscosity value if heated for a long period of time.

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The Synthesis of C^{14} Carbonyl Labeled Dimethyl Phthalate*

By DONALD Y. BARKER and JOHN E. CHRISTIAN

Special techniques and apparatus for the synthesis of C^{14} carbonyl labeled dimethyl phthalate, $C_6H_4(C_2CH_3)C^{14}_2CH_3$, are described.

DIMETHYL PHTHALATE is a compound showing considerable merit as a very effective insect repellent (1). In any study of the properties of this compound as an insect repellent, the use of a radioactive label would offer certain obvious advantages. Consideration of the structure of dimethyl phthalate, the ease of synthesis and the labeling isotopes available led to the conclusion that the C^{14} carbonyl labeled compound offered the most promise. Accordingly, the purpose of this study was to investigate the methods of synthesis of C^{14} carbonyl labeled dimethyl phthalate.

After careful consideration of various syntheses of dimethyl phthalate reported in the literature (2–5), C^{14}-labeled dimethyl phthalate was prepared by treatment of *o*-tolyl magnesium bromide with $C^{14}O_2$ (obtained from $BaC^{14}O_3$), oxidation of the resulting labeled *o*-toluic acid to labeled phthalic acid, and esterification via the acid chloride.

EXPERIMENTAL

***o*-Tolyl Magnesium Bromide.**—A twofold excess of this Grignard reagent was prepared using 3.42 Gm. of freshly distilled *o*-bromotoluene, 0.48 Gm. of magnesium turnings, one crystal of iodine and 30 ml. of anhydrous ether.

The magnesium turnings, previously crushed, were placed in a thoroughly dried 300-ml. three-necked round-bottomed flask, fitted with a mercury sealed stirrer, a 100-ml. dropping funnel and a Hopkin's condenser, fitted at its upper end with a drying tube filled with calcium chloride. Anhydrous ether, 6.5 ml., and a crystal of iodine were added to the flask. The *o*-bromotoluene was added to the dropping funnel and 20 drops of this undiluted halide were added to the flask. The mixture was refluxed without stirring, over a water bath at 45°. The remaining 23.5 ml. of anhydrous ether which had been used to rinse the apparatus in which the *o*-bromotoluene had been measured, was added to the dropping funnel. The *o*-bromotoluene-ether mixture was added with stirring to the flask over a thirty- to thirty-five-minute period. Stirring was continued as long as there was evidence of any reaction, after which the mixture was refluxed on the water bath for another thirty minutes.

***o*-Toluic Acid.**—The *o*-tolyl magnesium bromide was carbonated in the apparatus diagrammed in Fig. 1. Barium carbonate C^{14} (1.97 Gm.) with an activity of approximately 5 μc. was weighed into flask *G* and 15 ml. of concentrated sulfuric acid were added to funnel *F*. This carbon dioxide generator was attached and sealed to the manifold *L* through the drying tube *E*, filled with Drierite® and stopcock *D* was left open. The entire system was evacuated through stopcock *A* to less than 0.5 mm. pressure, as indicated by manometer *C*; stopcock *A* was closed and the system was tested for leaks. Stopcock *B* was opened and nitrogen admitted to bring the system to atmospheric pressure. Stopper *M* was opened and the *o*-tolyl magnesium bromide quickly pipetted into the reaction flask *K*. The stopper was quickly replaced, stopcock *B* was closed and the contents of the reaction flask frozen with liquid nitrogen and the system evacuated. Stop-

* Received August 28, 1954, from the Bio-Nucleonics Laboratory, School of Pharmacy, Purdue University, Lafayette, Ind.
1 resented to the Scientific Section of A. PH. A., Boston, meeting, August, 1954.
The C^{14}-labeled barium carbonate was obtained on allocation from the Atomic Energy Commission. Other materials were standard chemical reagents.

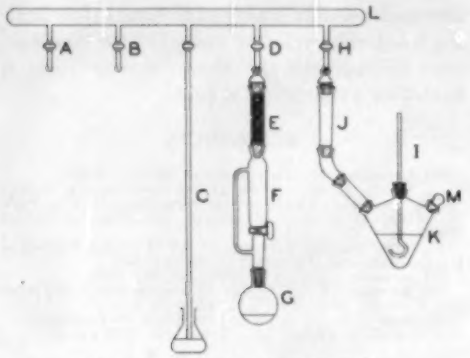

Fig. 1.—Apparatus for carbonation.

cock *A* was closed and the liquid nitrogen bath was replaced by an acetone-dry ice bath at −20°. The nitrogen entrapped in the reaction mixture escapes at this temperature. The *o*-tolyl magnesium bromide solution was again frozen with liquid nitrogen, the system evacuated, stopcock *A* closed and the temperature again raised to −20°. When the contents of the reaction flask had come to thermal equilibrium with the acetone-dry ice bath, stirrer *I* was started. Carbon dioxide was generated by carefully dropping the sulfuric acid onto the barium carbonate. Absorption of the carbon dioxide in flask *K* was noted by a constant manometer reading. To ensure complete absorption of the carbon dioxide, the contents of flask *K* were frozen with liquid nitrogen drawing any traces of carbon dioxide into flask *K*. Stopcock *H* was closed, the contents of the flask warmed to −20° and the mixture stirred for an additional fifteen minutes.

The carbonated *o*-tolyl magnesium bromide was hydrolyzed in the reaction flask by the addition of 20 Gm. of chipped ice, 100 ml. of distilled water, and 10 ml. of concentrated sulfuric acid. The liberated *o*-toluic acid dissolved in the ethereal layer on stirring. The mixture was transferred to a 500-ml. separatory funnel and the aqueous layer run off into a second funnel. This aqueous layer was extracted twice with two 25-ml. portions of ether, which had previously been used to rinse the reaction flask. The *o*-toluic acid was extracted from the ethereal solution using 60 ml. of 1 *N* sodium hydroxide solution in four equal portions. The ether fraction was discarded.

The combined aqueous fractions were acidified with hydrochloric acid, precipitating the *o*-toluic acid which was collected on Sharkskin filter paper.

Phthalic Acid.—The *o*-toluic acid was dissolved in 60 ml. of 1 *N* sodium hydroxide solution contained in a 250-ml. Erlenmeyer flask. To the solution was added 80 ml. of distilled water containing 3.48 Gm. of potassium permanganate, a 10% excess of the amount required to oxidize the theoretical amount of *o*-toluic acid.

The mixture was heated with constant stirring on a steam bath for ten hours. Excess potassium permanganate was reduced by the addition of 5 ml. of 95% ethyl alcohol. The solution was cooled to room temperature, acidified to litmus with concentrated hydrochloric acid and stirred again at steam bath temperature for thirty minutes. Sufficient saturated solution of sodium bisulfite (3 ml.) was added to the mixture to convert the precipitate of manganese dioxide to soluble manganese sulfate and the volume of the clear solution reduced to about 20 ml. by evaporation under a current of warm air. The solution was cooled in an ice bath, the phthalic acid allowed to crystallize and the crystals collected on Sharkskin filter paper. To the filtrate was added 0.2 Gm. of inactive phthalic acid. These crystals were collected on the first batch of crystals, recrystallized from water, collected, dried, and weighed. The C^{14} carbonyl labeled phthalic acid weighed 1.6 Gm., representing an over-all yield of 84.4% based on the C^{14} barium carbonate.

Dimethyl Phthalate.—The phthalic acid contained in a 125-ml. Erlenmeyer flask was converted to the acid chloride by the addition of 6.32 Gm. of phosphorus pentachloride, reaction being expedited by intimately mixing while heating over a small flame. On obtaining a clear solution, the flask was placed in an ice bath and the mouth of the flask fitted with a 25-cm. air condenser. Through the top of the condenser was added, in small portions, 5 ml. of absolute methyl alcohol, waiting after the addition of each portion for the reaction to subside.

Distilled water (5 ml.) containing 2 Gm. of sodium carbonate was added, and the contents transferred to a 125-ml. separatory funnel. The dimethyl phthalate was extracted using three 10-ml. portions of ether. The ether was removed by evaporation and the dimethyl phthalate was dried over calcium chloride for forty-eight hours. The dimethyl phthalate was then transferred to a 10-ml. distilling flask, the flask being washed with repeated 0.5-ml. portions of absolute methyl alcohol. The dimethyl phthalate was collected at 275–279° in a small tared ampul, capable of being hermetically sealed. The C^{14} carbonyl labeled dimethyl phthalate weighed 1.79 Gm. representing an over-all yield of 80.4% based on the C^{14} barium carbonate.

The purity of the C^{14} dimethyl phthalate was determined by paper partition chromatography. Sheets of Whatman Filter Paper No. 1 were cut into strips 30 × 3 cm. These strips were impregnated in a solution of acetone and formamide (70:30) for twelve hours after which the strips were dried for twenty-four hours at room temperature. The dimethyl phthalate to be chromatographed was applied on a line 8 cm. from the end. After nine hours of ascending chromatography using a solvent mixture consisting of 95% alcohol, water, and formic acid (49:49:2) an R_f value of 0.88 was obtained for both labeled and unlabeled dimethyl phthalate. Only one radioactive spot was found to be present on the paper. Dragendorff's reagent was employed to locate chemically the position of the dimethyl phthalate. This reagent reacted with the dimethyl phthalate producing a distinct yellow-brown spot.

SUMMARY AND CONCLUSIONS

1. A synthesis suitable for the preparation of C^{14} carbonyl labeled phthalic acid and dimethyl phthalate has been described.

2. The synthesis described has been used for the preparation of a quantity of C^{14} carbonyl labeled dimethyl phthalate with an over-all yield

of 80.4 per cent, based on the starting compound, C^{14} labeled barium carbonate.

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The Determination of Iodide-Iodate Activity in Sodium Radio-Iodide (I^{131}) by Automatic Scanning of Paper Chromatograms*

By JOHN J. PINAJIAN and JOHN E. CHRISTIAN

R_f values of different width filter paper chromatograms using 75 per cent methanol in an ascending system are presented for iodide and iodate. The chemical identity of the spots is determined by the use of carrier iodide and iodate. The iodide spot was developed by spraying the dry chromatogram with starch hydrogen peroxide solution. The iodate spot was developed with a spray of ascorbic acid-starch solution. Description of the equipment is given. The relationship of the chart drive speed and the optimal slit width to the time constant of the counting rate meter is discussed and the minimal slit width calculated for the instrument used. Integration of the curves gave areas representing the total activity at each spot. The ratio of the iodide-iodate activities thus determined differed from the relative readings of the counting rate meter.

IN THE COURSE of an investigation concerning the assay of Sodium Radio-Iodide (I^{131}) used for medicinal purposes,[1] it became necessary to distinguish between the iodide and iodate forms of the I^{131} activity present. Since the amount of material present for analysis represented something in the order of 10^{-12} Gm. ordinary techniques of differentiation were not suitable. The technique of paper chromatography appeared to be the most suitable and the most convenient instrument readily available for the resolution of the two forms of I^{131} activity. Indeed, once separated, the very nature of the iodide or iodate present allows for a fairly accurate estimation of the relative amounts present. The common practice of utilizing autoradiographic procedures with subsequent visual or densitometric determination of the amount of fogging was not suitable because of the time factor involved. Various direct methods of scanning have been described (2–7).

EXPERIMENTAL

Paper Chromatography.—A finely drawn capillary was inserted into the Sodium Radio-Iodide (I^{131})[1] and approximately one lambda was allowed to be drawn up by capillary action. This was then spotted one inch from the bottom of 18-inch strips of Whatman No. 1 filter paper (widths of 8 mm., 10 mm., and 20 mm. were used). The spots were air dried and a drop of carrier solution (using a standard dropper) was then spotted at the same site. The carrier solution contained 1.66 mg. potassium iodide, 2.14 mg. potassium iodate, and 10 mg. sodium bicarbonate per ml. The paper was air dried and a small strip of tin foil was folded over the bottom as a weight.

A 250-ml. ground-glass stoppered graduated cylinder was used for the chamber. Methanol, 25 ml. of 75 per cent,[2] was pipetted into the bottom and the strip was hung so that one-half inch of the tin foil weighted bottom was immersed in the solvent. The ground glass stopper held the paper in place. The chromatogram was allowed to develop for four hours during which time the solvent front traveled approximately 30 cm. The strip was withdrawn from the cylinder, the solvent front marked immediately, and the strip allowed to air dry.

Chemical Identification of the Spots.—The spots on the filter paper chromatograms were chemically identified by spraying the paper with a fine spray of Starch Test Solution, U. S. P. XIV, spraying with Hydrogen Peroxide Solution, U. S. P. XIV, and finally spraying with ascorbic acid solution, 5 per cent. The sprays were applied with a DeVilbiss No. 251 atomizer. The ascorbic acid solution was prepared fresh each month.

The spray of starch and hydrogen peroxide gave a rapid (less than one minute) response (brown color) with the iodide spot, while the iodate spot remained unaffected. With the addition of ascorbic acid, the iodate was reduced promptly (less than one minute) to give a purple color changing to brown within two minutes.

The spraying of starch-iodide impregnated paper with ascorbic acid gave negative results, even after

* Received August 28, 1954, from the Bio-Nucleonics Laboratory, School of Pharmacy, Purdue University, West Lafayette, Ind.
Presented to the Scientific Section of A. PH. A., Boston, meeting, August, 1954.
[1] Obtained from the Oak Ridge National Laboratory, Oak Ridge, Tenn.
[2] The solvent system used here was first suggested by Gleason (8), Abbott Laboratories, Oak Ridge, Tenn.

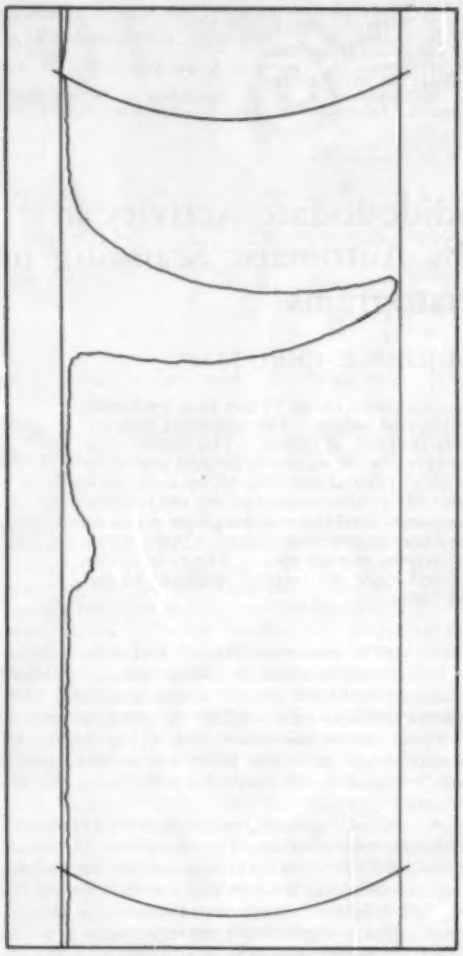

Fig. 1.—Recording of the iodide and iodate activities of a filter paper chromatogram of sodium iodide (I^{131}). (The lower most marking locates the spotting site and the upper most marking locates the solvent front line.)

one hour. The addition of hydrogen peroxide to the iodate spot after treatment with starch spray similarly gave negative results.

Radioactivity Assay of the Chromatograms.—For determining the activities of the iodide and iodate spots, the strips were pasted along the edge of a section of chart paper (Esterline-Angus Chart 4309-C), allowing alternating 30-cm. sections of clear chart paper in between. The chart paper was then rolled on to the spindle of a 1-ma. Esterline-Angus recorder. Small weights were clipped on the end of the chart paper. The chart paper was arranged so that the chromatogram passed in front of a thin slit of a brass plate which shielded the Geiger-Müller counter.[3] Constant geometry was maintained by placing a lead block in back of the chart paper. The pulses from the Geiger-Müller tube were fed to a counting rate meter[4] and from there to the recorder. As the paper chromatogram spotting line passed a line at the middle of the slit, the pen was manually moved across the chart to give a spotting line on the chart. Similarly, as the solvent front line moved across the marker, another line was made on the chart.

With the arrangement described, specification as to the chart drive speed and the optimal slit width is dependent upon the time constant of the counting rate meter. The value for the time constant varies in different circuits and sensitivities from one second to about one minute and is minimal for the highest counting rate sensitivity in a given circuit. Thus, it is advantageous to work whenever possible with a high level of activity in order to minimize the time constant of the rate meter response.[5]

When this is done, the practical limiting factors are the chart drive speed and the slit width. With a chart drive speed of 28 cm./hr., the minimal slit width was calculated to be 3.4 mm.

The slit width was machined to 5.0 mm. and was made 20 mm. across to accommodate various widths of filter paper strips.

The R_f values obtained under the conditions described were 0.76 ± 5% and 0.45 ± 5% for iodide and iodate, respectively, and were in correspondence with R_f values determined by the conventional method. The method described has the advantage of presenting well-defined peaks which represent the centers of density. Thus the uncertainty of the R_f value is considerably reduced by simple measurement of well-defined distances. A sample recording is reproduced in Fig. 1.

A planimeter was used to measure the area beneath the curves. This area represents the total number of counts in the spot. The background was subtracted by extending the horizontal base line. The distribution of the radioactivity between the iodide and iodate spots in the sample recording illustrated above was found to be 93.6 and 6.4% for iodide and iodate, respectively. The distribution as calculated from the relative readings of the counting rate meter was found to be 92.4 and 7.6% for iodide and iodate, respectively.

SUMMARY

A simple automatic scanning and recording device has been described for the I^{131} paper chromatography. The relationship of the chart drive speed and the optimal slit width to the time constant of the counting rate meter has been discussed and the minimal slit width has been calculated for the instrument used. R_f values of radioiodide filter paper chromatograms have been presented. A method for determining the ratio of the iodide-iodate activities present have been described. A method for the chemical identifica-

[3] End-window Geiger-Müller counter with a 2.3-mg./cm.² mica window.

[4] Model 1615, Radiation Sentinel, Nuclear Instrument and Chemical Corp., Chicago, Ill.

[5] One should not, of course, count at such a rate that resolving time corrections become necessary. For example, with the full-scale response of the counting rate meter set at 5000 c. p. m. and the resolving time of the Geiger-Müller counter 200 μ sec., the correction for losses at the maximum rate will be approximately 1.68 per cent. Since the counting rate varies from zero through the maximum to zero for a given spot, the resolving time error in the estimation of the activity of the spot will be less than 1.68 per cent.

tion of the iodide and iodate spots on the paper chromatograms has been developed.

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Properties and Applications of Powdered Polysaccharide Acids*

I. Preparation and Titration Curves

By JOSEPH V. SWINTOSKY,† LLOYD KENNON,‡ and JAMES TINGSTAD

Several new powdered polysaccharide acids, namely elm acid, quince acid, and plantago acid, were prepared by ion exchange, followed by spray drying. These acids, carboxymethylcellulose acid and several analogous type acids were titrated potentiometrically, and the shape of the titration curves were indicated to be quite typical of the synthetic cation exchangers. Approximate acid equivalents and pKa values were determined for some of these substances. A number of pharmaceutical applications for these acids are suggested.

BERRY AND RIDOUT have described the use of powdered alginic acid as a tablet disintegrant (1) and we have similarly noted and reported this property (2) for the spray-dried powdered acid form of carboxymethylcellulose (HCMC).

Some recent publications have also described the cation exchange properties of alginic acid (3, 4, 5) and chemically modified cotton fabrics (6). Thus a number of the naturally occurring gums and related products possess the chemical structural requirements for ion exchange.

Possible uses already discovered for some of the polysaccharide acids, coupled with other chemical and physical features, suggested a program embracing their preparation and an investigation of their pharmaceutical applications.

EXPERIMENTAL

Materials.—The polysaccharide acids employed in this study were derived from algin, sodium carboxymethylcellulose, linseed, elm, quince, acacia, and plantago seed. Alginic acid employed in this study was Kelacid®.[1] The powdered linseed acid and the arabic acid were prepared by ion exchange and spray drying in these laboratories, and have been previously described (2, 7). Powdered HCMC has also been prepared (2); but for this study, a slight modification of the previous method of preparation was followed.

Preparation of Elm Acid Solution.—Approximately 2.3 Kg. of elm bark[2] was used. Ten L. of boiling distilled water was poured over the bark contained in a porcelain pan. The water was allowed to cool to room temperature, and the mucilaginous layers formed on the bark surfaces were dispersed into the water by rubbing. The rubbing process was repeated after two hours of maceration. The liquid infusion was then separated from the bark. The bark was again subjected to the steeping process. About 20 L. of an orange-brown mucilaginous infusion of elm bark was obtained. The infusion was saturated with chlorobutanol to inhibit fermentation and was then strained through two layers of muslin. The mucilaginous polysaccharide was precipitated from solution by pouring 60 L. of alcohol, into the 20 L. of infusion. It appeared as a white fluffy substance somewhat like paper pulp and was collected by filtration through muslin. It was then expressed free of liquid by squeezing in the hands; the material became light ash colored. In 150 cc. of alcohol it assumed a very friable character and crumbled into small particles upon stirring. Distilled water was added in small increments with stirring to permit uniform wetting and hydration of the product. When a total volume of 1 L. was attained, the product, a thick homogeneous mucilage, was poured with stirring into 1 L. of boiling distilled water. After further swelling, it was diluted with warm distilled water to a total volume of 8 L. and after standing several hours, 24 L. of alcohol were added to reprecipitate the polysaccharide. The precipitate was filtered through muslin and redissolved in water to a volume of 20 L. The series of alcohol precipitations were performed to separate the polysaccharide gum from the other principal extractive materials of the bark.

To obtain the elm acid solution, the viscous gum solution, maintained at about 50–60°, was passed through a series of eight 4-L. flasks, each flask containing about 500 Gm. of acid charged IR-120 cation exchange resin.[3] About fifteen to twenty minutes

* Received April 22, 1954, from the School of Pharmacy, University of Wisconsin, Madison. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.
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[1] Kelco Co., Chicago, Ill.
[2] Elm bark, quince seed, and plantago seed were obtained from S. B. Penick Co., N. Y.
[3] Rohm & Haas sulfonic acid type resin.

were permitted for ion exchange in each flask. After shaking the flask several times on a hot plate, the ion exchange resin settled and the supernatant liquid was decanted into the next flask. The solution from flask 8 was, in each case, filtered through a single layer of muslin to remove the major portion of suspended IR-120 resin. The acid gum solution was a translucent colorless product of considerably lower viscosity than the solution prior to ion exchange.

Preparation of Quince Acid and Plantago Acid Solutions.—About 2 Kg. of quince seed, Alicante, was used to prepare the quince acid. About 350 Gm. of triple cleaned, ovata blonde, plantago seed was used to prepare the plantago acid. The principles and procedures employed for the preparation of these acid gum solutions were similar to those described for elm acid, although the separation of the mucilage from the seeds was less difficult than its removal from the elm bark.

Preparation of HCMC Solution.—Since the previously described method (2) for the preparation of HCMC solution by ion exchange was extremely slow, the method was modified to conform with that described for the preparation of elm acid solution. About 40 L. of a 2% aqueous solution (CMC, Type 70, Hercules Cellulose Gum,[4] premium low viscosity) were passed through the series of sulfonic acid resin charged flasks without the employment of heat.

Preparation of Powdered Polysaccharide Acids.—Preparatory to spray drying, the respective polysaccharide acid solutions were warmed to about 50°, and filtered through four layers of muslin. These cloudy colloidal solutions were then sprayed through an atomizing head into a 230° chamber of a Bowen Spray Drier according to previously described methods (2, 7).

Ash Determination.—Ash determinations were made by igniting the respective samples on crucibles, without any pretreatment, and weighing the residues.

Titration Curves of the Powdered Polysaccharide Acids.—Samples of the acids were titrated as collected from the spray drier. Samples of approximately 0.4 Gm. in 50 cc. of distilled water were titrated potentiometrically with 0.100 *N* NaOH using a Beckman Model H *p*H meter. A period of several minutes was permitted between additions of alkali for equilibration.

For one of these acids, HCMC, special precautions were taken to conduct the titration so that a more reliable value of acid strength and ion exchange capacity could be obtained. In this procedure numerous 0.400-Gm. samples of HCMC were placed in Erlenmeyer flasks, each containing 50 cc. of 1.00 *M* NaCl. To each flask, increments of 0.100 *N* NaOH varying by several cc. were added. The total volume of each flask was then brought to 100 cc. with freshly boiled distilled water, and the flasks were stoppered. After standing at room temperature for twenty-four hours, with occasional shaking, the *p*H's were determined.

RESULTS AND DISCUSSION

All of the newly prepared spray-dried polysaccharide acids were very fine powders, forming cloudy suspensions when shaken with water. In this respect they differed from arabic acid which dissolves in water to form clear colloidal solutions (7). Those polysaccharide acids derived from quince and plantago were light tan in color, while the product from elm was grayish-white. The HCMC was a white powder similar in appearance to the previously reported product (2), although there was a slight amount of charred particles due perhaps to slightly defective atomization in the spray drying procedure.

The ash values of these polysaccharide acids were as follows: linseed (2),[5] 0.67%; quince, 3.01%; plantago, 1.73%; elm, 8.4%; CMCA (2),[5] 0.78%; arabic (7),[5] 0.044%; alginic, 1.6%. These values suggest that conversion to the acid form was not entirely complete for those products prepared by ion exchange and spray drying. The elm acid, in particular, gave a relatively high ash value. Viscous products, such as the elm gum solution here used, presumably requires a much longer period for ion exchange equilibration than was provided in this procedure. The products must then be considered in the light of these properties and the methods employed in their preparation and not necessarily as pure entities.

The potentiometric titration curves of these respective products are illustrated in Fig. 1. The curves *A*, *B*, *C*, *D*, *E*, *F*, and *H* are direct titration curves determined without control of volume or establishing *p*H equilibrium after each addition of alkali. Thus they represent neutralization curves which indicate the approximate acid equivalents, and the comparative acid strengths of these respec-

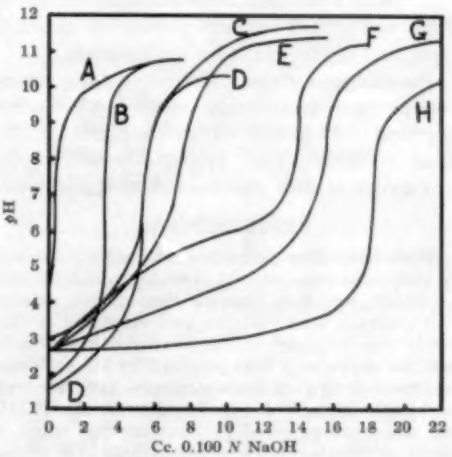

Fig. 1.—Potentiometric titration curves employing 0.400-Gm. samples of *A*, plantago acid; *B*, arabic acid; *C*, quince acid; *D*, linseed acid; *E*, elm acid; *F* and *G*, HCMC; and *H*, alginic acid. All curves except *G* were determined by suspending the powders in 50 cc. of distilled water, followed by immediate titration with 0.100 *N* NaOH. Curve *G* was obtained by permitting 24-hour equilibrium neutralization in 100 cc. of 0.50 *M* NaCl containing various appropriate amounts of NaOH.

[4] Hercules Powder Co., Wilmington, Del.

[5] Previously reported in references indicated.

tive substances. The curve patterns follow the form of the *p*H titration curves of resinous exchangers, as determined by Griessbach (14). These products owe their acidity to the presence of carboxyl groups (8–13); and direct titration curves are observed to have the single inflection shapes comparable to that observed by Griessbach and others (14–17) for carboxylic ion exchangers.

The acid equivalents for the respective polysaccharide acids, calculated from the direct titration curves were as follows: plantago, 10,000; arabic,[6] 1250; quince, 753; linseed,[7] 747; elm, 567; HCMC,[8] 286; alginic,[9] 221. These values indicate that alginic acid and HCMC possess the highest acid reserve and ion exchange capacity; and offer the most promise for applications where these properties are important. With powdered HCMC, the frequency of carboxyl groups in the molecule, molecule chain lengths, degree of dispersion, swelling, and solubility all apparently may be modified by the chemical preparative procedure from cellulose.

A more satisfactory method for determining the acid strength and ion exchange capacity of a polyfunctional high molecular weight acid as a function of *p*H is to carry out the titration in an excess of neutral salt. This procedure has been followed in some of the studies of Topp and Pepper (15), and Gregor and Bregman (16). These workers have shown for the resinous cation exchangers that in the presence of excess neutral salt the relation of capacity to *p*H can be determined directly from the titration curve, giving results comparable to those that would be obtained if the exchangers were in true solution. The same relationship would be expected with the high molecular weight polysaccharide acids. When HCMC is allowed to react with the neutral salt, NaCl, equilibrium results.

$$\text{HCMC} + \text{NaCl} \rightleftarrows \text{Na}^+\,\text{CMC}^- + \text{HCl}$$

The equilibrium is shifted to the right as the *p*H is increased until all of the HCMC has been converted to the sodium salt. Thus curve *G*, where the neutralization titration is conducted in the presence of excess neutral salt, under equilibrium conditions and constant volume, assumes the shape, and displays a sharp inflection, comparable to that of a relatively strong monobasic acid in homogeneous solution. Under these conditions clear solutions resulted when the *p*H was on the alkaline side and cloudy suspensions remained when the *p*H was on the acid side. In comparing the HCMC titration curves *F* and *G*, we note in curve *G*, a displacement to lower *p*H values in the presence of neutral salt, indicating a higher ion exchange capacity at the lower *p*H values when the neutral salt is present. This is in keeping with similar results observed from studies of other carboxylic ion exchangers (6, 15, 16).

The method employed for curve *G* permits a closer estimation of total ion exchange capacity of powdered HCMC than is possible from curve *F*. The total ion exchange capacity would correspond to the amount of sodium combined with the ion exchanger at the point of inflection of curve *G*, at about *p*H 8.4. This gives an ion exchange capacity of about 3.9 m. e. q. per gram.

The *p*H of half neutralization corresponds very closely to the point of half maximum exchange capacity and therefore is a close approximation of the pKa. This gives pKa = 4.1 for this product, a value in close agreement with that observed for carboxymethylcellulose fabrics, acetic acid, succinic acid, and some similar carboxylic acids (6).

Our experiences with the powdered polysaccharide acids have suggested a number of avenues for further study. For instance, studies dealing with their value in removal of exogenous sodium from the intestinal tract are indicated. Their apparent innocuous character when taken internally, and the property of imparting a *p*H of 2–3 to aqueous suspensions suggests a use in treating gastric hypoacidity. Their value as enteric coating materials, tablet disintegrants, or retarders of tablet disintegration, their utility in the production of effervescent tablets, and in sustained-release drug therapy seem worthy of investigation. They may be of use in rendering more palatable fluid alkaloidal preparations or other drugs, by removing all or a portion of the distasteful materials from solution. When employed in pharmaceutic dosage forms, the *p*H effect might be utilized to inhibit bacterial growth. Ability to sustain an acidic *p*H without being absorbed may be useful in the development of spermatocides, or in the treatment of local infections occurring in certain types of vaginitis. A number of these possible fields of application for the powdered polysaccharide acids have been under study in our laboratories and will be reported at later dates.

SUMMARY

1. Several new powdered polysaccharide acids from quince, elm, and plantago have been prepared by ion exchange of the corresponding gum solutions with a sulfonic acid exchanger, followed by spray drying of these solutions. Direct potentiometric titration curves of these acids and HCMC, alginic acid, linseed acid, and arabic acid illustrate their comparative acid strengths and acid equivalents; their analogy to titration curves of synthetic cation exchangers is indicated.

2. A potentiometric titration curve of HCMC in neutral salt solution has been used to calculate the approximate ion exchange capacity and pKa of this substance. The ion exchange capacity of HCMC at *p*H 8.4 was observed to be about 3.9 m. e. q. per gram, and the pKa about 4.1.

3. A number of possible pharmaceutical applications for polysaccharide acids have been suggested.

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Properties and Applications of Powdered Polysaccharide Acids*

II. Tablet Studies

By JOSEPH V. SWINTOSKY,† LLOYD KENNON,‡ and JAMES TINGSTAD

Several new powdered polysaccharide acids were tested for their effects on the hardness and disintegration times of sulfathiazole tablets. Their effects were compared with those of some better known additives in tablet formulas. Several of these new polysaccharide type acids were shown to hasten disintegration whereas others retarded disintegration.

ALGINIC ACID has been described in the scientific literature as a tablet disintegrant (1); and some proprietary tablets in pharmaceutical commerce employ alginic acid for this purpose. We have similarly noted and reported tablet disintegrant properties (2) for the powdered acid form of carboxymethylcellulose (HCMC).

In previous papers from these laboratories (2, 3, 4) the preparation of several new powdered polysaccharide acids by ion exchange and spray drying has been described. This paper illustrates the use of these substances in a sulfathiazole formulation; and shows their effect on disintegration and hardness of the tablets.

EXPERIMENTAL

Materials.—Alginic acid employed in this study was Kelacid,[1] a commercial brand. The linseed acid, HCMC, arabic acid, elm acid, quince acid, and plantago acid, were prepared in these laboratories (4).

Procedure.—One tablet lot was prepared according to the following formula:

	Composition, %
Sulfathiazole...	97.0
Starch.....	3.0 (added as a 10% paste)

The other tablet lots were prepared according to the formula:

	Composition, %
Sulfathiazole...	93.0
Polysaccharide..	4.0
Starch.....	3.0 (added as a 10% paste)

Since quantities utilized for this study were small, somewhat greater amounts of starch paste binder were used than is customary in tablet granulation procedures. This was done in order to facilitate uniform mixing of the components on an ointment slab with use of a spatula. Where dry starch was used, the starch powder was added after suitable dry gram les had been prepared from the sulfathiazole and starch paste mixture. With the plantago acid the wet mixing was done in a glass mortar, since the mass possessed an exceptionally sticky consistency.

The masses were passed through a U. S. #12 screen and dried at 110°. The dried granules were passed through a U. S. #16. These granular materials were employed in the preparation of 0.33 Gm., 3/8-inch diameter flat face tablets.

Ten tablets were prepared at compressional forces of 1,000, 1,500, 2,000, 2,500, and 3,000 pounds per tablet. The machine used for this purpose was described previously (5). Five tablets at each compressional force were tested for disintegration rate in distilled water, using a modified U. S. P. method described previously (6). All tests were accomplished within two weeks following preparation of the tablets.

RESULTS AND DISCUSSION

Tabulations on tablet hardness and disintegration rate appear in Tables I and II. In practically all instances, when median hardness values at each compressional force are considered, the magnitude of these values increases with compressional force, through the range of forces employed. Tablet hardness does not vary markedly from one formula to another at any given force level except for the formula devoid of disintegrant, and that with the plantago acid. The tablets without disintegrant appear softer, while those containing plantago acid appear harder than the other products.

For all the formulations, except the one employing alginic acid, the tablet disintegration time increases significantly with increasing compressional force

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[1] Kelco Co., Chicago, Ill.

TABLE I.—MINIMUM, MEDIAN, AND MAXIMUM HARDNESS OF FIVE 0.33-GM. 3/8-INCH DIAMETER, FLAT FACE SULFATHIAZOLE TABLETS AS DETERMINED ON STRONG-COBB TESTER[a]

Compressional Force, lb./tablet	No Disintegrant (Control)	Contain 4% of Additive Indicated						
		Starch (Control)	Alginic Acid	HCMC	Elm Acid	Arabic Acid	Quince Acid	Linseed Acid	Plantago Acid
1000	5.8	7.4	9.9	6.2	6.4	8.0	6.9	6.0	8.0
	6.2	8.0	13.1	6.8	8.2	8.2	7.4	6.9	12.5
	8.0	10.7	14.6	12.5	11.2	8.9	9.3	9.5	17.0
1500	9.5	8.2	12.3	10.0	9.5	10.3	10.4	8.9	16.0
	10.2	9.7	14.4	10.9	13.1	11.8	11.7	11.3	16.7
	10.5	11.0	20.0	12.6	13.6	17.3	13.0	12.5	17.3
2000	8.0	13.0	13.0	13.8	11.8	12.9	13.7	14.3	20.0
	11.8	15.9	18.9	14.0	15.9	16.4	17.3	18.3	21.5
	16.7	20.5	22.5	18.3	18.3	20.0	17.7	19.5	24.0
2500	15.1	10.9	17.2	12.4	18.5	13.1	15.0	17.0	18.1
	16.0	17.1	23.4	14.3	24.3	19.6	18.9	19.2	21.3
	19.0	20.0	24.3	23.2	24.4	24.4	23.9	24.8	26.7
3000	8.3	11.4	17.7	19.3	22.2	17.8	17.8	17.4	25.1
	12.0	20.4	22.0	19.5	26.6	19.7	22.0	24.0	29.0
	18.0	27.4	25.9	28.8	29.5	27.8	23.1	27.4	30.0

[a] Median values are underlined.

(Table II). The data also illustrate the relative merit of each of the polysaccharide acids as a tablet disintegrant. Where a sulfathiazole tablet devoid of disintegrant is used as a control it is apparent from the median disintegration time values that alginic acid, HCMC, elm acid, and starch serve to facilitate tablet disintegration. The utility of elm acid for this purpose heretofore has not been reported. The efficiency of HCMC in promoting tablet disintegration confirms a previous observation made in these laboratories (2).

SUMMARY

1. It is evident from the disintegration rate data, for the tablets containing linseed acid, quince acid, arabic acid, and the plantago acid, that these substances do not facilitate disintegration of sulfathiazole tablets. In fact, these substances are disintegration retarders in this tablet formulation.

2. The tablets containing the plantago acid became gummy and sticky immediately upon immersion in water in contrast to the other tablets; and those prepared at 1,000 pounds force did not show signs of disintegration even after 20,000 seconds. Because the plantago acid exhibited properties akin to some of the common pharmaceutical gums, further studies on the effects of this substance on tablet disintegration were not continued.

3. These data suggest that by (*a*) judicious selection of disintegrant, (*b*) suitable choice of tablet composition, and (*c*) regulation of the com-

TABLE II.—MINIMUM, MEDIAN, AND MAXIMUM DISINTEGRATION TIMES (SEC.) OF FIVE 0.33-GM. 3/8-INCH DIAMETER, FLAT FACE SULFATHIAZOLE TABLETS IN WATER AT 37°, USING MODIFIED U. S. P. METHOD[a]

Compressional Force, lb./tablet	No Disintegrant (Control)	Contain 4% of Additive Indicated							
		Starch (Control)	Alginic Acid	HCMC	Elm Acid	Arabic Acid	Quince Acid	Linseed Acid	Plantago Acid
1000	336	241	333	143	478	7898	330	737	20,000
	569	437	443	168	509		526	1011	
	711	726	494	212	681		950	1085	
1500	874	629	240	260	416	14816	2749	3361	
	1031	726	414	318	472		4294	3673	
	1150	1071	615	369	694		9689	4110	
2000	1078	690	297	593	1142		12301	4600	
	2089	1001	313	779	1150		13506	6357	
	2364	2242	352	1152	1335		16191	6664	
2500	911	1040	245	440	1362			7744	
	1960	1629	329	954	1543			9361	
	3385	2983	345	1288	1784			10604	
3000	1983	890	423	583	1916			9386	
	3122	2155	434	1405	2283				
	5218	3480	478	1596	2321				

[a] Median values are underlined.

pressional force to precise and known values, the rates of tablet disintegration and drug release may be controlled. Starch has been employed widely because rapid disintegration rate has been considered a desirable quality in most pharmaceutical tablets. Now such products as HCMC and elm acid may have similar application in promoting rapid tablet disintegration. An apparent advantage over starch is their inherent acid buffering capacity in tablet formulations where the *p*H effect is important.

4. In recent years the concepts of sustained drug release, repeat drug release, and delayed drug release have become popular and a need has arisen for tablet additives and designs which allow for such types of drug release over periods of hours. Disintegration retarders or inhibitors such as arabic acid, linseed acid, and quince acid may be possibly employed in these new applications where delayed or sustained drug release is desired.

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The Use of Some Imitation Flavors for Masking Distasteful Drugs*

V. Aureomycin Hydrochloride

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The efficacy of some imitation flavored concentrates prepared in the laboratory as masking agents for the initial and obnoxious tastes of aureomycin hydrochloride was studied. When compared with some of the official syrups some were found to disguise this drug satisfactorily.

IT WAS THE PURPOSE of this investigation to determine the effectiveness of certain imitation flavors, as masking agents for aureomycin hydrochloride.

EXPERIMENTAL

Preparation of Imitation Flavored Concentrates.—The following imitation flavored concentrates were used in this study: grape, root beer, butterscotch, maple, raspberry, cream soda, wild cherry, coconut, and ginger ale. With the exception of ginger ale, the formulas and methods of preparations as well as some evaluation of their masking power were given in previous papers (1–3).

The formula for the imitation ginger ale concentrate appears below:

Imitation Ginger Ale Concentrate	
Oleoresin of Ginger, N. F. IX	1.14
Oleoresin of Capsicum (High pungency), N. F. IX	0.29
Lemon Oil	0.19
Oil of Lime	0.75
Oil of Rose (Rosanlik)	0.0019
Oil of Estragon	0.0019
Alcohol, a sufficient quantity, to make	100

Macerate the ingredients in the alcohol for seven days, then filter until clear, using talc if necessary.

Preparation of Syrups.—The imitation flavored syrups were prepared as previously described (1), i. e., by adding 1.67 ml. of the concentrate to enough simple syrup to make 1000 ml. The acidified syrups of grape, raspberry, and wild cherry were prepared by adding 1.67 ml. of the concentrate and 4.00 Gm. of citric acid dissolved in 2 ml. of distilled water to enough simple syrup to make 1000 ml.

The official syrups compared with the imitation flavored syrups were citric acid, U. S. P., orange, U. S. P., cacao, U. S. P., cherry, U. S. P., and raspberry, U. S. P.

Concentrations of Aureomycin Hydrochloride Taste Test Preparations.—The concentrations of this antibiotic used for the taste tests ranged from a minimum of subliminal amount to a maximum containing at least a therapeutic amount in each 30 ml. of preparation.

The subliminal concentration is defined as that amount of drug that cannot be detected by taste and was determined on a series of solutions of the drug by means of taste tests conducted with a panel. The method of conducting the taste tests will be mentioned later.

* Received August 27, 1954, from the College of Pharmacy, University of Florida, Gainesville.
Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.
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Seventeen concentrations of aureomycin hydrochloride[1] were used. A concentration three dilutions below the subliminal concentration was selected as the lowest concentration. The concentrations ranged from 0.39 mg. to 259.9 mg. of drug in each 30 ml. of solution, increasing proportionally so that a ratio of 1.1:5 existed between adjacent solutions. They were numbered from 1 to 17 in ascending order of concentration so that the data obtained from the taste tests could be statistically analyzed (Table I). This method of analysis has been previously described (1, 4).

TABLE I.—CONCENTRATIONS OF AUREOMYCIN HYDROCHLORIDE USED IN TASTE TESTS

Number of Taste Test	Concentration of Drug in 4-ml. Taste Test, mg.	Concentration of Drug in 30-ml. Preparation, mg.
1	0.05	0.39
2	0.08	0.59
3	0.12	0.89
4	0.18	1.34
5	0.27	2.00
6	0.40	3.00
7	0.60	4.50
8	0.90	6.75
9	1.35	10.14
10	2.03	15.21
11	3.04	22.82
12	4.56	34.23
13	6.85	51.35
14	10.27	77.03
15	15.40	115.55
16	23.11	173.33
17	34.63	259.9

When using antibiotics in taste testing a wider range of concentrations is required than with compounds having a single, pure taste, such as salt or bitter, that is clearly distinguished in solution. It was found in this study that aureomycin hydrochloride was extremely astringent as well as bitter.

The vehicular solutions and suspensions were prepared in the same manner as were the aqueous except that distilled water was replaced by a vehicular solution consisting of one part of flavored syrup, or simple syrup, diluted with five parts of water, so that the resulting concentrations of drug would correspond with those listed in Table I. Thus, the only difference between any taste tests of the same number would be the vehicle. This permitted a direct comparison of the vehicles with the aqueous preparations of the drug and, also, with every other vehicle.

Taste Tests—The Method of Evaluation of the Vehicles.—The masking efficiencies were determined by taste tests conducted with panels and the data statistically analyzed. The taste test panels consisted of at least fifteen adults, both male and female, in apparent good health and not affected by conditions which could interfere with the senses of taste and smell. They were untrained except for detailed instructions given them just prior to the taste testing and during the entire time the testing was being conducted. The subjects were divided into groups of 4 to 6 persons each in order that each individual could be closely supervised and observed throughout the tests. The taste tests were conducted in the same manner and the data statistically treated as previously described by the authors and others (1, 4).

The results of the panel testing are given in Tables II–V. Tables II and IV give the significances of the mean disguising and mean obnoxious potentials of the vehicles as masking agents for aureomycin hydrochloride. The disguising potential of a vehicle is the difference between the taste threshold of the drug in water and in the vehicle. The aqueous taste

TABLE II.—THE SIGNIFICANCES OF THE MEAN DISGUISING POTENTIALS OF SOME IMITATION FLAVORED AND OFFICIAL VEHICLES FOR SP. AUREOMYCIN HYDROCHLORIDE

Vehicle	Number of Subjects, n	Mean Threshold in Water ± Standard Error, $m_2 \pm e_2$	Mean Threshold in Vehicle ± Standard Error, $m_1 \pm e_1$	Mean Disguising Potential ± Standard Error, $M \pm e$	Significance of Difference, D
Cacao syrup, U. S. P.	15	4.53 ± 0.58	11.07 ± 0.29	6.54 ± 0.67	6.97
Citric acid syrup, U. S. P.	15	4.27 ± 0.54	9.00 ± 0.32	4.73 ± 0.61	7.28
Cherry syrup, U. S. P.	15	4.27 ± 0.54	8.87 ± 0.40	4.60 ± 0.46	6.87
Orange syrup, U. S. P.	15	4.27 ± 0.56	8.60 ± 0.40	4.33 ± 0.43	6.29
Imitation coconut syrup	16	5.50 ± 0.63	9.69 ± 0.43	4.19 ± 0.63	5.51
Imitation raspberry syrup	17	5.76 ± 0.53	9.88 ± 0.31	4.12 ± 0.43	6.75
Imitation grape syrup, acid added	15	6.00 ± 0.61	10.00 ± 0.44	4.00 ± 0.50	6.00
Imitation grape syrup	16	5.56 ± 0.59	9.56 ± 0.48	4.00 ± 0.46	5.26
Imitation butterscotch syrup	15	5.60 ± 0.63	9.60 ± 0.42	4.00 ± 0.34	5.26
Imitation wild cherry syrup	16	5.50 ± 0.70	9.38 ± 0.45	3.88 ± 0.49	4.60
Imitation root beer syrup	16	5.69 ± 0.57	9.56 ± 0.40	3.87 ± 0.33	5.61
Imitation maple syrup	16	5.63 ± 0.66	9.50 ± 0.40	3.87 ± 0.48	5.03
Imitation wild cherry syrup, acid added	16	5.56 ± 0.65	9.13 ± 0.49	3.57 ± 0.65	4.40
Imitation cream soda syrup	16	5.63 ± 0.58	8.94 ± 0.54	3.31 ± 0.31	4.19
Imitation raspberry syrup, acid added	15	5.73 ± 0.59	8.73 ± 0.43	3.00 ± 0.39	4.11
Simple syrup, U. S. P.	16	5.63 ± 0.54	8.56 ± 0.48	2.93 ± 0.42	4.07
Raspberry syrup, U. S. P.	17	5.65 ± 0.37	8.53 ± 0.29	2.88 ± 0.35	6.13
Imitation ginger ale syrup	17	5.65 ± 0.37	8.35 ± 0.21	2.70 ± 0.19	6.28

[1] Supplied by Lederle Laboratories, Division Cyanamid Co., New York, N. Y.

TABLE III.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN DISGUISING POTENTIALS OF IMITATION FLAVORED AND OFFICIAL VEHICLES LISTED IN TABLE II FOR AUREOMYCIN HYDROCHLORIDE

Cacao Syrup, U. S. P.	Citric Acid Syrup, U. S. P.	Cherry Syrup, U. S. P.	Orange Syrup, U. S. P.	Imitation Coconut Syrup	Imitation Raspberry Syrup	Imitation Grape Syrup, Acid Added	Imitation Grape Syrup	Imitation Butterscotch Syrup	Imitation Wild Cherry Syrup	Imitation Root Beer Syrup	Imitation Maple Syrup	Imitation Wild Cherry Syrup, Acid Added	Imitation Cream Soda Syrup	Imitation Raspberry Syrup, Acid Added	Simple Syrup, U. S. P.	Raspberry Syrup, U. S. P.	Imitation Ginger Ale Syrup
1.99	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.40	0.17	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.76	0.54	0.43	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.55	0.62	0.53	0.18	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.03	0.81	0.76	0.34	0.09	..	..	..	..	..	..	..	..	..	..	..	..	..
3.02	0.92	0.88	0.50	0.23	0.18	..	..	..	..	..	..	..	..	..	..	..	..
3.14	0.95	0.92	0.53	0.24	0.19	0	..	..	..	..	..	..	..	..	..	..	..
3.39	1.04	1.05	0.60	0.27	0.22	0	0	..	..	..	..	..	..	..	..	..	..
3.20	1.08	1.07	0.69	0.39	0.37	0.17	0.18	0.20	..	..	..	..	..	..	..	..	..
3.56	1.25	1.28	0.85	0.45	0.46	0.22	0.23	0.28	0.02	..	..	..	..	..	..	..	..
3.20	1.10	1.09	0.72	0.41	0.38	0.19	0.20	0.22	0.01	0	..	..	..	..	..	..	..
3.19	1.30	1.29	0.97	0.68	0.71	0.52	0.54	0.59	0.38	0.41	0.37	..	..	..	..	..	..
4.36	*2.09*	*2.35*	1.92	1.26	1.53	1.17	1.25	1.50	0.98	1.24	0.98	0.36	..	..	..	..	..
4.54	*2.40*	*2.67*	*2.29*	1.61	1.93	1.56	1.67	1.96	1.40	1.71	1.40	0.75	0.62	..	..	..	..
4.57	*2.43*	*2.69*	*2.33*	1.66	1.98	1.65	1.73	1.98	1.48	1.77	1.47	0.83	0.73	0.12	..	..	..
4.82	*2.64*	*2.97*	*2.64*	1.79	*2.25*	1.84	1.93	*2.29*	1.67	*2.06*	1.65	0.93	0.93	0.23	0.09	..	..
5.49	*3.17*	*3.80*	*3.47*	*2.26*	*3.02*	*2.25*	*2.60*	*3.33*	*2.27*	*3.08*	*2.25*	1.28	1.69	0.70	0.50	0.45	0

TABLE V.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN CHNOXIOUS POTENTIALS OF IMITATION FLAVORED AND OFFICIAL VEHICLES LISTED IN TABLE IV FOR AUREOMYCIN HYDROCHLORIDE

Cacao Syrup, U. S. P.	Citric Acid Syrup, U. S. P.	Cherry Syrup, U. S. P.	Orange Syrup, U. S. P.	Imitation Coconut Syrup	Imitation Maple Syrup	Imitation Wild Cherry Syrup	Imitation Ginger Ale Syrup	Imitation Cream Soda Syrup	Raspberry Syrup, U. S. P.	Imitation Grape Syrup, Acid Added	Imitation Raspberry Syrup	Imitation Butterscotch Syrup	Imitation Root Beer Syrup	Imitation Wild Cherry Syrup, Acid Added	Imitation Grape Syrup	Simple Syrup, U. S. P.	Imitation Raspberry Syrup, Acid Added
1.76	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1.72	0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.11	0.55	0.54	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.37	0.74	0.73	0.14	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.69	1.03	1.00	0.37	0.24	..	..	..	..	..	..	..	..	..	..	..	..	..
2.82	1.10	1.04	0.39	0.26	0	..	..	..	..	..	..	..	..	..	..	..	..
3.26	1.34	1.27	0.48	0.33	0.03	0.04	..	..	..	..	..	..	..	..	..	..	..
3.27	1.38	1.34	0.53	0.40	0.10	0.12	0.11	..	..	..	..	..	..	..	..	..	..
3.29	1.40	1.35	0.54	0.41	0.12	0.13	0.13	0.03	..	..	..	..	..	..	..	..	..
2.85	1.22	1.19	0.56	0.44	0.21	0.23	0.23	0.16	0.15	..	..	..	..	..	..	..	..
3.36	1.58	1.48	0.69	0.58	0.31	0.33	0.40	0.30	0.28	0.05	..	..	..	..	..	..	..
3.32	1.58	1.51	0.79	0.69	0.43	0.48	0.56	0.47	0.45	0.19	0.19	..	..	..	..	..	..
3.32	1.61	1.57	0.86	0.75	0.77	0.56	0.65	0.57	0.55	0.30	0.30	0.11	..	..	..	..	..
3.38	1.20	1.63	0.88	0.79	0.55	0.60	0.95	0.62	0.60	0.31	0.33	0.14	0.02	..	..	..	..
3.93	*2.34*	*2.28*	1.49	1.45	1.24	1.34	1.61	1.53	1.51	1.00	1.19	0.95	0.80	0.81	..	..	..
4.56	*2.88*	*2.73*	1.74	1.74	1.53	1.71	*2.50*	*2.31*	*2.29*	1.22	1.70	1.26	1.02	1.04	0	..	..
4.10	*2.52*	*2.45*	1.64	1.59	1.41	1.52	1.86	1.78	1.76	1.16	1.42	1.13	0.97	0.97	0.15	0.20	0

TABLE IV.—THE SIGNIFICANCES OF THE MEAN OBNOXIOUS POTENTIALS OF SOME IMITATION FLAVORED AND OFFICIAL VEHICLES FOR AUREOMYCIN HYDROCHLORIDE

Vehicle	Number of Subjects, n	Mean Threshold in Water ± Standard Error, $m_2 \pm \sigma_2$	Mean Threshold in Vehicle ± Standard Error, $m_1 \pm \sigma_1$	Mean Obnoxious Potential ± Standard Error, $M \pm \epsilon$	Significance of Difference, D
Cacao syrup, U. S. P.	15	9.07 ± 0.64	13.73 ± 0.27	4.66 ± 0.64	6.67
Citric acid syrup, U. S. P.	15	8.93 ± 0.64	12.13 ± 0.50	3.20 ± 0.53	3.95
Cherry syrup, U. S. P.	15	8.93 ± 0.64	12.13 ± 0.53	3.20 ± 0.56	3.85
Orange syrup, U. S. P.	15	8.93 ± 0.64	11.67 ± 0.57	2.74 ± 0.64	3.19
Imitation coconut syrup	16	9.94 ± 0.61	12.56 ± 0.41	2.62 ± 0.57	3.54
Imitation maple syrup	16	9.94 ± 0.60	12.38 ± 0.49	2.43 ± 0.53	3.12
Imitation wild cherry syrup	16	10.13 ± 0.60	12.56 ± 0.58	2.43 ± 0.46	2.93
Imitation ginger ale syrup	17	8.35 ± 0.41	10.76 ± 0.36	2.41 ± 0.26	4.38
Imitation cream soda syrup	16	9.44 ± 0.47	11.81 ± 0.39	2.37 ± 0.27	3.90
Raspberry syrup, U. S. P.	17	8.35 ± 0.41	10.71 ± 0.32	2.36 ± 0.27	4.54
Imitation grape syrup, acid added	15	10.33 ± 0.51	12.60 ± 0.53	2.27 ± 0.54	3.07
Imitation raspberry syrup	17	9.88 ± 0.38	12.12 ± 0.32	2.24 ± 0.33	4.48
Imitation butterscotch syrup	15	10.13 ± 0.47	12.27 ± 0.39	2.14 ± 0.41	3.49
Imitation root beer syrup	16	9.81 ± 0.40	11.88 ± 0.36	2.07 ± 0.45	3.82
Imitation wild cherry syrup, acid added	16	10.00 ± 0.60	12.06 ± 0.56	2.06 ± 0.42	2.51
Imitation grape syrup	16	10.43 ± 0.39	12.00 ± 0.45	1.56 ± 0.46	2.64
Simple syrup, U. S. P.	16	9.93 ± 0.40	11.50 ± 0.46	1.56 ± 0.22	2.56
Imitation raspberry syrup, acid added	15	9.86 ± 0.42	11.33 ± 0.29	1.46 ± 0.44	2.86

threshold is that level or concentration at which the drug may be detected by taste as compared with a control of distilled water containing no drug. The vehicular threshold is that concentration at which the drug is detected in the vehicle. The obnoxious threshold is simply that concentration at which the drug is considered as undesirable for oral administration. It can be seen in Tables II and IV that all vehicles, both imitation flavored and official, were significantly better than water for masking the initial and obnoxious tastes of aureomycin hydrochloride.

Tables III and V show the results of a comparison of each vehicle with all others used in the study as masking agents for the initial and obnoxious tastes of aureomycin hydrochloride. The italicized values indicate a significant difference between the respective vehicles. In Table III it is seen that no imitation flavored vehicle studied here was significantly better than simple syrup for masking the initial taste of aureomycin hydrochloride. Raspberry and butterscotch syrups and imitation root beer syrup were significantly better than raspberry syrup, U. S. P. No vehicle, imitation flavored or official, except cacao syrup, U. S. P. was significantly better than these three imitation flavored syrups and they did not differ significantly among themselves. The addition of citric acid to the imitation fruit flavored syrups, raspberry, wild cherry, and grape did not significantly alter the mean disguising potentials of these vehicles.

The values in Table V show that imitation flavored ginger ale and cream soda syrups were significantly better than simple syrup for masking the obnoxiousness of aureomycin hydrochloride. Of the official vehicles all except orange syrup were better than simple syrup, however, with the exception of cacao syrup, U. S. P., none of the official vehicles was superior to these two imitation flavored vehicles. While lowering the mean obnoxious potentials for all of the imitation fruit flavored vehicles, the addition of citric acid did not significantly depress them.

DISCUSSION AND CONCLUSIONS

In this study all vehicles, imitation flavored and official, were significantly better than water for masking both the initial and obnoxious tastes of aureomycin hydrochloride. While no imitation flavored vehicle was significantly better than simple syrup for masking the taste of this drug, imitation ginger ale and cream soda syrups were superior to simple syrup. Cacao syrup was the only official syrup better than these two imitation vehicles. Imitation raspberry, butterscotch, and root beer syrups were significantly better than simple syrup for masking the initial taste of aureomycin hydrochloride and only cacao syrup, U. S. P., was superior to these three vehicles. It is interesting to note that while the five vehicles having the highest potentials in Table II also had the highest potentials in Table IV, there seems to be no other particular pattern of relationship among the others for the respective vehicles. Thus, the fact that a vehicle has a high mean disguising potential does not necessarily indicate that it will also have a high mean obnoxious potential.

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The Use of Some Imitation Flavors for Masking Distasteful Drugs*

VI. Penicillin G Potassium

By BETTY LANKFORD MCLAUGHLIN† and CHARLES H. BECKER‡

Imitation root beer, cream soda, and ginger ale syrups were found to be the best of all vehicles, imitation flavored and official, as masking agents for penicillin G potassium. Cacao syrup, U. S. P., the only official vehicle used in this investigation, was not significantly superior to any vehicle.

IN THE PREVIOUS STUDY (1) it was found that some imitation flavored syrups when compared with some official syrups proved satisfactory vehicles for the antibiotic aureomycin hydrochloride, which is distasteful. As penicillin G potassium is administered orally to a great degree, and is quite distasteful, this investigation was continued by studying the effectiveness of these same vehicles, except those that were acidic, as masking agents for this second antibiotic.

EXPERIMENTAL

In this investigation the imitation flavored and official vehicles, taste test solutions and controls, and method of evaluating the vehicles were the same as those used in the aureomycin hydrochloride (1) study, except that all acidic vehicles were omitted here due to the instability of penicillin preparations at a low *p*H. Liquacillin® diluent, Eli Lilly's buffered diluent used for penicillin G potassium, was included among the vehicles in this study.

Concentrations of Penicillin G Potassium Taste Test Solutions.—Nineteen concentrations of this antibiotic (Penicillin G potassium,[1] crystalline, buffered), ranging from 0.89 mg. to 1,316.22 mg., or 1,335 to 1,974,000 U. S. P. units, respectively, per 30 ml. of solution were used in this study. A ratio of 1:1.5 existed between each adjacent strength. The taste tests were numbered 1 to 19, in ascending order of concentration. The drug was soluble in all concentrations used. In Table I are given the numbers of the solutions of the drug with the corresponding concentrations, in milligrams and in U. S. P. units, of penicillin G potassium in a 4-ml. taste test and, also, in a 30-ml. preparation.

Results of the Taste Tests.—In Tables II and IV it is seen that all vehicles, imitation flavored and official, were significantly better than water for masking the initial taste of penicillin G potassium and that all of the vehicles, except imitation coconut, were also better than water for masking the obnoxiousness of this drug.

Results given in Table III show that no imitation flavored or official vehicle was significantly better than simple syrup for masking the initial taste of penicillin G potassium. Imitation root beer and cream soda syrups were superior to cacao syrup, U. S. P., the only official vehicle studied. This official syrup had a lower mean disguising potential than did simple syrup and was not significantly better than any of the imitation flavored syrups. Imitation root beer syrup was superior to seven of the vehicles, cream soda superior to five, and grape superior to three of the vehicles. These three did not vary significantly among themselves.

In Table V it is seen that no vehicle, imitation flavored or official, was significantly better than syrup for masking the obnoxiousness of penicillin G potassium. With the exception of imitation maple syrup, the five vehicles having the highest mean obnoxious potentials were significantly better than imitation raspberry and coconut syrups. There were no significant differences among these five vehicles with high mean obnoxious potentials.

DISCUSSION AND CONCLUSIONS

All vehicles studied here were significantly better than water as a vehicle for penicillin G potassium,

TABLE I.—CONCENTRATIONS OF PENICILLIN G POTASSIUM USED IN TASTE TESTS

Number of Taste Test	Concentration of Drug in 4-ml. Taste Test		Concentration of Drug in 30-ml. Preparation	
	mg.	U. S. P. Units	mg.	U. S. P. Units
1	0.12	176	0.89	1,335
2	0.18	267	1.34	2,010
3	0.27	400	2.00	3,000
4	0.40	600	3.00	4,500
5	0.60	900	4.50	6,750
6	0.90	1,350	6.75	10,125
7	1.35	2,025	10.14	15,210
8	2.03	3,045	15.21	22,815
9	3.04	4,560	22.82	34,230
10	4.56	6,840	34.23	51,345
11	6.85	10,275	51.35	77,017
12	10.27	15,405	77.03	115,545
13	15.40	23,100	115.55	173,325
14	23.11	34,665	173.33	259,995
15	34.65	51,975	259.99	389,850
16	51.99	77,985	389.93	584,850
17	77.99	116,985	584.99	877,350
18	116.99	175,485	877.48	1,316,100
19	175.48	263,220	1,316.22	1,974,000

* Received August 27, 1954, from the College of Pharmacy, University of Florida, Gainesville.
Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.
Based upon a dissertation submitted by Betty Lankford McLaughlin to the Graduate School of the University of Florida, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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[1] Supplied by Bristol Laboratories Inc., Syracuse, N. Y.

TABLE II.—THE SIGNIFICANCES OF THE MEAN DISGUISING POTENTIALS OF SOME IMITATION FLAVORED AND OFFICIAL VEHICLES[a] FOR PENICILLIN G POTASSIUM

Vehicle	Number of Subjects, n	Mean Threshold in Water ± Standard Error, $m_2 \pm \sigma_2$	Mean Threshold in Vehicle Standard Error, $m_1 \pm \sigma_1$	Mean Obnoxious Potential Standard Error, $M \pm \sigma$	Significance of Difference, D
Imitation root beer syrup	15	7.87 ± 0.71	13.80 ± 0.26	5.93 ± 0.56	7.80
Imitation cream soda syrup	15	8.20 ± 0.66	14.07 ± 0.20	5.87 ± 0.62	8.51
Imitation grape syrup	18	8.83 ± 0.65	13.94 ± 0.42	5.11 ± 0.50	5.11
Simple syrup, U. S. P.	16	9.50 ± 0.65	14.06 ± 0.49	4.56 ± 0.49	5.63
Liquacillin® diluent (Lilly)	15	8.93 ± 0.52	13.47 ± 0.22	4.54 ± 0.46	8.11
Imitation maple syrup	16	9.13 ± 0.61	13.63 ± 0.43	4.50 ± 0.36	6.00
Imitation wild cherry syrup	16	8.56 ± 0.75	12.94 ± 0.38	4.38 ± 0.68	5.15
Cacao syrup, U. S. P.	15	8.93 ± 0.52	13.13 ± 0.29	4.20 ± 0.45	7.12
Imitation raspberry syrup	16	9.31 ± 0.57	13.50 ± 0.41	4.19 ± 0.49	5.98
Imitation butterscotch syrup	20	9.00 ± 0.50	12.80 ± 0.49	3.80 ± 0.32	5.43
Imitation coconut syrup	19	9.95 ± 0.49	12.42 ± 0.39	3.47 ± 0.40	5.48
Imitation ginger ale syrup	15	8.93 ± 0.52	12.40 ± 0.49	3.47 ± 0.41	4.82

[a] All vehicles acid in nature were omitted due to the instability of penicillin in solutions at a low pH.

TABLE III.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN DISGUISING POTENTIALS OF THE IMITATION FLAVORED AND OFFICIAL VEHICLES LISTED IN TABLE II FOR PENICILLIN G POTASSIUM[a]

Imitation Root Beer Syrup	Imitation Cream Soda Syrup	Imitation Grape Syrup	Simple Syrup, U. S. P.	Liquacillin® Diluent (Lilly)	Imitation Maple Syrup	Imitation Wild Cherry Syrup	Cacao Syrup, U. S. P.	Imitation Raspberry Syrup	Imitation Butterscotch Syrup	Imitation Coconut Syrup	Ginger Ale Syrup
0.07	..	..	..	..	..	..	..	..	..	..	..
1.09	0.96	..	..	..	..	..	..	..	..	..	..
1.83	1.66	0.79	..	..	..	..	..	..	..	..	..
1.93	1.73	0.84	0.03	..	..	..	..	..	..	..	..
2.17	1.90	1.00	0.10	0.07	..	..	..	..	..	..	..
1.76	1.62	0.87	0.21	0.20	0.16	..	..	..	..	..	..
2.40	*2.17*	1.36	0.54	0.53	0.52	0.22	..	..	..	..	..
2.35	*2.13*	1.31	0.53	0.52	0.51	0.23	0.02	..	..	..	..
3.33	*2.96*	*2.22*	1.29	1.32	1.46	0.77	0.73	0.66	..	..	..
3.57	*3.24*	*2.56*	1.70	1.75	1.91	1.15	1.22	1.14	0.65	..	..
3.57	*3.24*	*2.56*	1.70	1.75	1.91	1.15	1.22	1.14	0.65	0	0

[a] The italicized values indicate a significant difference between respective vehicles.

TABLE IV.—THE SIGNIFICANCES OF THE MEAN OBNOXIOUS POTENTIALS OF SOME IMITATION FLAVORED AND OFFICIAL VEHICLES[a] FOR PENICILLIN G POTASSIUM

Vehicle	Number of Subjects, n	Mean Threshold in Water Standard Error, $m_2 \pm \sigma_2$	Mean Threshold in Vehicle Standard Error, $m_1 \pm \sigma_1$	Mean Obnoxious Potential Standard Error, $M \pm \sigma$	Significance of Difference, D
Imitation root beer syrup	15	13.73 ± 0.62	16.00 ± 0.32	2.27 ± 0.36	3.24
Imitation cream soda syrup	15	13.60 ± 0.66	15.87 ± 0.29	2.27 ± 0.46	3.15
Liquacillin® diluent (Lilly)	15	12.87 ± 0.43	15.13 ± 0.04	2.26 ± 0.36	5.02
Imitation maple syrup	16	13.63 ± 0.66	15.63 ± 0.33	2.00 ± 0.46	2.70
Imitation ginger ale syrup	15	12.87 ± 0.43	14.80 ± 0.28	1.93 ± 0.25	3.78
Imitation grape syrup	18	14.28 ± 0.47	16.00 ± 0.32	1.72 ± 0.39	3.02
Imitation butterscotch syrup	20	13.95 ± 0.59	15.65 ± 0.32	1.70 ± 0.40	2.54
Cacao syrup, U. S. P.	15	12.87 ± 0.43	14.53 ± 0.28	1.66 ± 0.30	3.26
Imitation wild cherry syrup	16	13.75 ± 0.46	15.25 ± 0.41	1.50 ± 0.45	2.42
Simple syrup, U. S. P.	16	14.56 ± 0.39	15.88 ± 0.39	1.32 ± 0.36	2.54
Imitation raspberry syrup	16	14.63 ± 0.35	15.69 ± 0.36	1.06 ± 0.28	2.12
Imitation coconut syrup	19	14.84 ± 0.41	15.63 ± 0.31	0.84 ± 0.46	1.65

[a] All vehicles acid in nature were omitted due to the instability of penicillin in solutions at a low pH.

with the exception of imitation coconut for the obnoxiousness of this drug. While no vehicle, imitation flavored or official, was better than simple syrup for masking either the initial or repugnant tastes of this drug, imitation root beer and cream soda syrups were superior to a number of the vehicles, including cacao syrup, U. S. P., for disguising both of these effects. While the Liquacillin® diluent had a low mean disguising potential, it was superior to two of the vehicles for masking the odious taste of penicillin G potassium. In general, there were shown no particular relationships between the mean disguising potentials of the vehicles and their respective mean obnoxious potentials. In fact, imitation ginger ale

TABLE V.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN OBNOXIOUS POTENTIALS OF THE IMITATION FLAVORED AND OFFICIAL VEHICLES LISTED IN TABLE IV FOR PENICILLIN G POTASSIUM[a]

Imitation Root Beer Syrup	Imitation Cream Soda Syrup	Liquacillin® Diluent (Lilly)	Imitation Maple Syrup	Imitation Ginger Ale Syrup	Imitation Grape Syrup	Imitation Butterscotch Syrup	Cacao Syrup, U. S. P.	Imitation Wild Cherry Syrup	Simple Syrup, U. S. P.	Imitation Raspberry Syrup	Imitation Coconut Syrup
0	..	..	..	..	..	..	..	..	..	..	..
0.02	0.02	..	..	..	..	..	..	..	..	..	..
0.47	0.42	0.45	..	..	..	..	..	..	..	..	..
0.77	0.65	0.75	0.13	..	..	..	..	..	..	..	..
1.04	0.90	1.02	0.46	0.46	..	..	..	..	..	..	..
1.06	0.93	1.04	0.49	0.49	0.04	..	..	..	..	..	..
1.30	1.11	1.28	0.62	0.69	0.12	0.08	..	..	..	..	..
1.33	1.20	1.31	0.78	1.04	0.37	0.33	0.30	..	..	..	..
1.86	1.64	1.84	1.17	1.39	0.75	0.72	0.74	0.31	..	..	..
2.69	*2.24*	*2.67*	1.74	*2.29*	1.38	1.31	1.43	0.83	0.58	..	..
2.24	*2.17*	*2.41*	1.76	*2.10*	1.47	1.41	1.49	1.03	0.81	0.41	0

[a] The italicized values indicate a significant difference between the respective vehicles.

syrup, which had the lowest mean disguising potential, was found to have one of the five highest mean obnoxious potentials and was significantly better than two vehicles.

REFERENCE

(1) McLaughlin, B. L., and Becker, C. H., THIS JOURNAL 44, 114(1955).

The Use of Some Imitation Flavors for Masking Distasteful Drugs*

VII. Terramycin® Hydrochloride

By BETTY LANKFORD McLAUGHLIN† and CHARLES H. BECKER‡

The imitation flavored vehicles found best for masking Terramycin® hydrochloride were wild cherry, plain, and acid added, root beer, and ginger ale syrups. Several found excellent for masking the initial taste of this drug were not found as effective for masking the obnoxious effects of it, particularly within the 5 per cent confidence levels. Cacao syrup, U. S. P. was significantly better than most vehicles, imitation flavored and official, for masking both effects of Terramycin® hydrochloride.

IN EARLIER PAPERS (1, 2) it was reported that some imitation flavored vehicles were as effective, and in some cases superior to, some official vehicles for masking the distastefulness of some antibiotics, aureomycin hydrochloride, and penicillin G potassium, which are frequently administered orally, and which are distasteful. Therefore, these investigations were continued and these same vehicles were evaluated as masking agents for the antibiotic Terramycin® hydrochloride, which also is frequently administered orally, and also is quite distasteful.

TABLE I.—CONCENTRATIONS OF TERRAMYCIN® HYDROCHLORIDE USED IN TASTE TESTS

Number of Taste Test	Concentration of Drug in 4-ml. Taste Test		Concentration of Drug in 30-ml. Preparation	
	890 μg./mg.	Pure	890 μg./mg.	Pure
1	0.06	0.05	0.44	0.39
2	0.09	0.08	0.66	0.59
3	0.13	0.12	0.99	0.89
4	0.20	0.18	1.50	1.34
5	0.30	0.27	2.24	2.00
6	0.45	0.40	3.36	3.00
7	0.67	0.60	5.04	4.50
8	1.02	0.90	7.57	6.75
9	1.52	1.35	11.39	10.14
10	2.28	2.03	17.09	15.21
11	3.42	3.04	25.64	22.82
12	5.13	4.56	38.46	34.23
13	7.69	6.85	57.70	51.35
14	11.54	10.27	86.55	77.03
15	17.31	15.40	129.83	115.55
16	25.97	23.11	194.75	173.33
17	38.9	34.7	292.0	259.9

* Received August 27, 1954, from the College of Pharmacy, University of Florida, Gainesville.
Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.
Based upon a dissertation submitted by Betty Lankford McLaughlin to the Graduate School of the University of Florida, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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‡ Associate Professor of Pharmacy, University of Florida, Gainesville.

TABLE II.—THE SIGNIFICANCES OF THE MEAN DISGUISING POTENTIALS OF SOME IMITATION FLAVORED AND OFFICIAL VEHICLES FOR TERRAMYCIN® HYDROCHLORIDE

Vehicle	Number of Subjects, n	Mean Threshold in Water ± Standard Error, $m_2 \pm e_2$	Mean Threshold in Vehicle ± Standard Error, $m_1 \pm e_1$	Mean Disguising Potential ± Standard Error, $M \pm e$	Significance of Difference D
Cacao syrup, U. S. P.	16	5.88 ± 0.60	12.31 ± 0.46	6.43 ± 0.54	8.46
Imitation wild cherry syrup, acid added	15	4.53 ± 0.57	9.80 ± 0.45	5.27 ± 0.41	7.22
Imitation root beer syrup	15	4.40 ± 0.50	9.53 ± 0.43	5.13 ± 0.50	7.77
Imitation wild cherry syrup	15	4.07 ± 0.37	9.13 ± 0.53	5.06 ± 0.34	7.78
Imitation grape syrup	15	4.33 ± 0.45	9.20 ± 0.47	4.87 ± 0.51	7.49
Raspberry syrup, U. S. P.	15	6.47 ± 0.52	10.80 ± 0.77	4.33 ± 0.33	4.66
Cherry syrup, U. S. P.	17	6.18 ± 0.57	10.41 ± 0.51	4.23 ± 0.50	5.49
Imitation coconut syrup	17	4.53 ± 0.57	8.24 ± 0.49	3.71 ± 0.51	4.95
Citric acid syrup, U. S. P.	17	5.88 ± 0.54	9.53 ± 0.63	3.65 ± 0.42	4.40
Imitation cream soda syrup	16	3.94 ± 0.39	7.56 ± 0.42	3.62 ± 0.51	6.37
Simple syrup, U. S. P.	16	5.38 ± 0.72	8.88 ± 0.57	3.50 ± 0.35	3.80
Imitation ginger ale syrup	15	6.47 ± 0.52	9.80 ± 0.44	3.33 ± 0.40	4.90
Orange syrup, U. S. P.	16	5.88 ± 0.60	9.19 ± 0.71	3.31 ± 0.54	3.60
Imitation grape syrup, acid added	15	5.73 ± 0.59	9.00 ± 0.47	3.27 ± 0.52	4.51
Imitation butterscotch syrup	16	4.62 ± 0.55	7.75 ± 0.56	3.13 ± 0.47	4.01
Imitation maple syrup	17	5.12 ± 0.32	8.18 ± 0.36	3.06 ± 0.37	6.19
Imitation raspberry syrup	17	5.67 ± 0.59	8.82 ± 0.57	3.06 ± 0.58	3.73
Imitation raspberry syrup, acid added	16	5.75 ± 0.62	8.50 ± 0.50	2.75 ± 0.58	3.27

TABLE IV.—THE SIGNIFICANCES OF THE MEAN OBNOXIOUS POTENTIALS OF SOME IMITATION FLAVORED AND OFFICIAL VEHICLES FOR TERRAMYCIN® HYDROCHLORIDE

Vehicle	Number of Subjects, n	Mean Threshold in Water ± Standard Error, $m_2 \pm e_2$	Mean Threshold in Vehicle ± Standard Error, $m_1 \pm e_1$	Mean Obnoxious Potential ± Standard Error, $M \pm e$	Significance of Difference, D
Cacao syrup, U. S. P.	16	9.69 ± 0.50	14.75 ± 0.51	5.06 ± 0.53	7.13
Cherry syrup, U. S. P.	17	10.06 ± 0.38	13.12 ± 0.37	3.06 ± 0.25	5.77
Raspberry syrup, U. S. P.	15	10.00 ± 0.64	12.87 ± 0.64	2.87 ± 0.78	3.15
Imitation ginger ale syrup	15	10.00 ± 0.64	12.80 ± 0.52	2.80 ± 0.47	3.41
Imitation wild cherry syrup	15	9.27 ± 0.37	11.87 ± 0.53	2.60 ± 0.54	4.00
Imitation root beer syrup	15	9.53 ± 0.57	12.13 ± 0.51	2.60 ± 0.41	3.42
Imitation wild cherry syrup, acid added	15	9.40 ± 0.38	11.93 ± 0.38	2.53 ± 0.43	4.69
Simple syrup, U. S. P.	16	9.75 ± 0.46	12.13 ± 0.42	2.38 ± 0.42	3.84
Citric acid syrup, U. S. P.	17	9.82 ± 0.45	12.18 ± 0.58	2.36 ± 0.37	3.23
Imitation maple syrup	17	9.18 ± 0.57	11.59 ± 0.53	2.31 ± 0.32	2.97
Imitation coconut syrup	17	9.29 ± 0.53	11.41 ± 0.57	2.12 ± 0.51	2.72
Imitation raspberry syrup, acid added	16	10.19 ± 0.70	12.19 ± 0.60	2.00 ± 0.49	2.17
Orange syrup, U. S. P.	16	9.81 ± 0.47	11.75 ± 0.72	1.94 ± 0.50	2.28
Imitation grape syrup	15	9.60 ± 0.57	11.53 ± 0.53	1.93 ± 0.50	2.54
Imitation raspberry syrup	17	9.94 ± 0.67	11.71 ± 0.60	1.77 ± 0.36	1.97
Imitation butterscotch syrup	16	8.06 ± 0.36	9.69 ± 0.55	1.63 ± 0.43	2.50
Imitation grape syrup, acid added	15	10.00 ± 0.55	11.47 ± 0.49	1.47 ± 0.38	1.99

EXPERIMENTAL

The imitation flavored and official vehicles, taste test solutions and controls, and method of evaluating the vehicles are the same as used in the study of Aureomycin hydrochloride (1).

Concentrations of Terramycin® Hydrochloride Taste Test Preparations.—Seventeen concentrations of Terramycin® hydrochloride[1] (chemical potency 890 μg./mg.) were used ranging from 0.44 mg. to 292.02 mg. of drug in each 30-ml. preparation. A ratio of 1:1.5 existed between each adjacent strength, and the tests were numbered from 1 to 17, respectively in ascending order of concentration. The drug was not completely soluble in higher concentrations and suspensions were used. As in the Aureomycin hydrochloride study (1) suspending agents were not added as the drug remained suspended for a long enough time after agitation for the taste tests to be conducted. The numbers of the solutions or suspensions with the corresponding concentrations, in milligrams, of Terramycin® hydrochloride, and of the pure drug, contained in a 4-ml. taste test and, also, in a 30-ml. preparation are given in Table I.

[1] Supplied by Chas. Pfizer & Co., Inc., New York, N. Y.

TABLE III.—THE SIGNIFICANCES OF DIFFERENCES BETWEEN THE MEAN DISGUISING POTENTIALS OF THE IMITATION FLAVORED AND OFFICIAL VEHICLES LISTED IN TABLE II FOR TERRAMYCIN® HYDROCHLORIDE[a]

Cacao Syrup, U. S. P.	Imitation Wild Cherry Syrup, Acid Added	Imitation Root Beer Syrup	Imitation Wild Cherry Syrup	Imitation Grape Syrup	Raspberry Syrup, U. S. P.	Cherry Syrup, U. S. P.	Imitation Coconut Syrup	Citric Acid Syrup, U. S. P.	Imitation Cream Soda Syrup	Simple Syrup, U. S. P.	Imitation Ginger Ale Syrup	Orange Syrup, U. S. P.	Imitation Grape Syrup, Acid Added	Imitation Butterscotch Syrup	Imitation Maple Syrup	Imitation Raspberry Syrup	Imitation Raspberry Syrup, Acid Added
1.71	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1.76	0.02	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.14	0.40	0.11	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.08	0.62	0.36	0.31	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.33	1.80	1.33	1.35	0.89	..	..	..	..	..	..	..	..	..	..	..	..	..
2.97	1.60	1.27	1.38	0.89	0.61	..	..	..	..	..	..	..	..	..	..	..	..
3.63	*2.40*	2.00	*2.21*	1.61	1.02	0.73	..	..	..	..	..	..	..	..	..	..	..
4.09	*2.75*	*2.28*	*2.67*	1.85	1.28	0.89	0.09	..	..	..	..	..	..	..	..	..	..
3.75	*2.54*	*2.13*	*2.36*	1.74	1.16	0.86	0.13	0.05	..	..	..	..	..	..	..	..	..
4.58	*3.28*	*2.67*	*3.25*	*2.21*	1.43	1.20	0.34	0.27	0.19	..	..	..	..	..	..	..	..
4.63	*3.40*	*2.81*	*3.33*	*2.37*	1.92	1.41	0.58	0.55	0.45	0.32	..	..	..	..	..	..	..
4.05	*3.27*	*2.46*	*2.74*	*2.08*	1.59	1.24	0.53	0.50	0.41	0.30	0.03	..	..	..	..	..	..
4.21	*3.03*	*2.58*	*2.89*	*2.19*	1.74	1.33	0.60	0.57	0.48	0.36	0.09	0.06	..	..	..	..	..
4.65	*3.45*	*2.94*	*3.39*	*2.52*	*2.11*	1.62	0.84	0.84	0.71	0.64	0.33	0.25	0.20	..	..	..	..
5.18	*4.02*	*3.33*	*4.00*	*2.87*	*2.59*	1.89	1.03	1.05	0.89	0.86	0.50	0.38	0.33	0.12	..	..	..
4.27	*3.11*	*2.69*	*2.99*	*2.35*	1.90	1.52	0.84	0.82	0.73	0.65	0.39	0.32	0.22	0.09	0	..	..
4.66	*3.55*	*3.09*	*3.45*	*2.75*	*2.37*	1.92	1.25	1.25	1.30	1.10	0.83	0.70	0.67	0.51	0.45	0.38	0

[a] The italicized values indicate the significant difference between the respective vehicles.

TABLE V.—THE SIGNIFICANCES OF THE DIFFERENCES BETWEEN THE MEAN OBNOXIOUS POTENTIALS OF THE IMITATION FLAVORED AND OFFICIAL VEHICLES LISTED IN TABLE IV FOR TERRAMYCIN® HYDROCHLORIDE[a]

Cacao Syrup, U. S. P.	Cherry Syrup, U. S. P.	Raspberry Syrup, U. S. P.	Imitation Ginger Ale Syrup	Imitation Wild Cherry Syrup	Imitation Root Beer Syrup	Imitation Wild Cherry Syrup, Acid Added	Simple Syrup, U. S. P.	Citric Acid Syrup, U. S. P.	Imitation Maple Syrup	Imitation Cream Soda Syrup	Imitation Coconut Syrup	Imitation Raspberry Syrup, Acid Added	Orange Syrup, U. S. P.	Imitation Grape Syrup	Imitation Raspberry Syrup	Imitation Butterscotch Syrup	Imitation Grape Syrup, Acid Added
3.39	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2.33	0.23	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.18	0.49	0.08	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.24	0.92	0.28	0.28	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.67	0.96	0.31	0.32	0	..	..	..	..	..	..	..	..	..	..	..	..	..
3.72	1.08	0.38	0.42	0.32	0.12	..	..	..	..	..	..	..	..	..	..	..	..
4.00	1.[illegible]	0.55	0.67	0.32	0.37	0.25	..	..	..	..	..	..	..	..	..	..	..
4.22	1.[illegible]	0.59	0.73	0.37	0.44	0.30	0.04	..	..	..	..	..	..	..	..	..	..
4.44	1.83	0.67	0.86	0.46	0.69	0.41	0.13	0.10	..	..	..	..	..	..	..	..	..
4.32	1.84	0.72	0.92	0.54	0.64	0.49	0.23	0.21	0.13	..	..	..	..	..	..	..	..
3.97	1.65	0.81	0.99	0.65	0.74	0.61	0.39	0.38	0.32	0.21	..	..	..	..	..	..	..
4.25	1.93	0.95	1.18	0.82	0.94	0.82	0.59	0.59	0.53	0.41	0.17	..	..	..	..	..	..
4.27	2.00	1.00	1.25	0.88	1.02	0.89	0.68	0.68	0.63	0.50	0.25	0.09	..	..	..	..	..
4.29	2.02	1.01	1.26	0.89	1.03	0.91	0.69	0.69	0.64	0.52	0.26	0.10	0.14	..	..	..	..
5.14	*2.93*	1.28	1.75	1.28	1.54	1.33	1.11	1.16	1.13	0.94	0.56	0.38	0.27	0.26	..	..	..
5.04	*2.83*	1.39	1.83	1.41	1.64	1.45	1.25	1.28	1.28	1.09	0.73	0.57	0.47	0.45	0.25	..	..
5.52	*3.53*	1.61	*2.22*	1.71	2.02	1.83	1.60	1.77	1.68	1.47	0.97	0.85	0.75	0.75	0.58	0.28	0

[a] The italicized values indicate the significant difference between the respective vehicles.

Results of Taste Tests.—Tables II and IV show that all vehicles were significantly better than water for masking the initial taste of Terramycin® hydrochloride and that all except imitation raspberry and grape, acid added, syrups were more effective than water for masking the obnoxiousness of this drug.

For masking the initial taste of Terramycin® hydrochloride, Table III shows that imitation root beer, and wild cherry, plain, and acid added, were significantly better than simple syrup, as well as superior to a number of the other vehicles, both imitation and official. In addition to these, imitation flavored grape proved a better vehicle than a number of other vehicles. No imitation flavored vehicle was significantly better than the official cherry and raspberry syrups. The addition of citric acid to the imitation fruit flavors significantly lowered the mean disguising potential of grape, but did not significantly affect those of raspberry and wild cherry. Cacao Syrup, U. S. P. was significantly better than all vehicles, except wild cherry, acid added, and root beer. Of the other official syrups, cherry, citric acid, and orange were not better than any vehicle, including simple syrup. Raspberry syrup, U. S. P., also, was not better than simple syrup, but was better than the three vehicles having the lowest mean disguising potentials.

Table V shows that no vehicle, imitation flavored or official, except cacao syrup, U. S. P., was significantly better than simple syrup. Imitation ginger ale syrup was superior to imitation grape, acid added, for masking the obnoxiousness of Terramycin® hydrochloride, this being the only observed significant difference between any of the imitation flavored vehicles. Imitation wild cherry, plain, and acid added, root beer, and ginger ale syrups were better, with differences within the 10 per cent level, than some other vehicles. No significant effects were produced by adding citric acid to the imitation fruit flavored syrups. Cacao syrup, U. S. P. was significantly better than all vehicles, and cherry syrup, U. S. P. superior to a few vehicles, for disguising the obnoxiousness of this drug. It can be thus seen that very few vehicles showed significant differences among themselves in this table.

DISCUSSION AND CONCLUSIONS

All vehicles in this study were superior to water for masking the initial taste of Terramycin® hydrochloride and all except imitation raspberry and grape, acid added, syrups were more effective than water for masking the obnoxiousness of this antibiotic. While imitation root beer, wild cherry, plain and acid added, and grape syrups were good vehicles for masking the initial taste of this drug, none of these were significant within the 5 per cent level for masking its unpleasantness. These first two were significant between the 5 and 10 per cent levels for masking the obnoxiousness of this drug, however. On the whole, the official vehicles were not found to be good, except cacao syrup, U. S. P., for Terramycin® hydrochloride. In this study it was found that the addition of citric acid to imitation grape flavor significantly lowered its mean disguising potential but did not alter the mean disguising potentials or mean obnoxious potentials of the others. In previous studies on these vehicles for other antibiotics (1, 2) citric acid did not significantly affect any of the potentials. While cacao syrup, U. S. P. had the highest mean disguising and obnoxious potentials, there were not any other observed relationships among the other vehicles for Terramycin® hydrochloride.

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Effect of Carbonic Anhydrase Inhibitors and Antacids on the Development of Gastrointestinal Ulcers during Treatment with Depot-Histamine*

A Preliminary Report

By JOS. W. E. HARRISSON,† E. W. PACKMAN,‡ P. S. GUTH, N. BACK, and W. S. CHERNICK

Depot-histamine-induced gastrointestinal ulceration in guinea pigs was used as the criterion for the evaluation of antiulcer effect. Dihydroxy aluminum sodium carbonate and dihydroxy aluminum aminoacetate were found to be the most effective of the antacids tested. Two powerful carbonic anhydrase inhibitors proved to be ineffective.

POTENTIAL ANTIULCER DRUGS are usually examined for their buffering, coating, antisecretory ability, and absence of acid-rebound effect. Gastrointestinal ulceration is, however, a complex phenomenon in which factors such as vasospasm, antipepsin content, and mucous secretion of the mucosa are involved. A good antacid is not necessarily a good antiulcer agent. The ultimate test of antiulcer activity is, of course, clinical usefulness. Some of the difficulties and pitfalls of clinical testing of antisecre-

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a The italicized values indicate the significant difference between the respective vehicles.

tory and antiulcer agents have been discussed by Rowlands, *et al.* (1). The method of Shay, *et al.* (2) for the production of gastric ulcers in rats has recently been criticized by Madden, *et al.* (3), and others. These investigators believe that the pathogenesis of acute ulceration in the stomach of the pylorus-ligated rat of Shay, *et al.*, is unrelated to that of clinical gastrointestinal ulcers. On the other hand this method may be a good indicator of acid secretion and thus suitable for the testing of antisecretory drugs.

It is a frequent observation that animals surviving for a period of time after the administration of toxic doses of histamine, often exhibit gastrointestinal ulceration. If guinea pigs are pretreated with antihistamines, then injected with extremely large doses of histamine, gastrointestinal ulcers will develop within twenty-four hours and the animals may die of gastrointestinal perforation and peritonitis. The antihistamines do protect against the respiratory, cardiovascular, etc., effects of histamine but are ineffective against its gastric-secretion-stimulating as well as gastrointestinal ulcer-inducing effect (4–6). Thus they prolong the life of the animal sufficiently to develop ulcers. Hay, *et al.* (7), demonstrated that daily injections of histamine in oil and beeswax cause gastrointestinal ulceration in a wide variety of species, the histologic picture of which is quite similar to human ulcers.

The aim of the present study was (*a*) to investigate the usefulness of the procedure of Hay, *et al.* (7), in testing the antiulcer effect of a number of well-known antacids and in addition a new antacid—dihydroxy aluminum sodium carbonate (DASC), (*b*) to study the possible antiulcer effect of two new potent carbonic anhydrase inhibitors, Diamox® (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) (6763) (American Cyanamide C.),[1] and Dirnate® (*p*-Carboxybenzene sulfonamide) (Sharp & Dohme, Division of Merck & Co.).[2]

Davenport and associates (8, 9) demonstrated the presence of carbonic anhydrase in the gastric mucosa, particularly in the parietal cells and indicated its possible role in gastric acid secretion. This was confirmed by Davies (10). A number of weak carbonic anhydrase inhibitors were found to be ineffective in inhibiting gastric acid secretion, until the new powerful inhibitors, Diamox® and Dirnate® became available. Janowitz, *et al.* (11), could inhibit histamine-induced acid secretion in the Heidenhain pouch dog with Diamox®. Birnbaum, *et al.* (12), demonstrated inhibition of the secretion of pancreatic juice (volume and bicarbonate content) by Diamox®. Presently Diamox® and Dirnate® are mainly used in therapeutics as diuretics and to increase sodium and potassium excretion.

METHODS

English smooth hair male guinea pigs of 250–300 Gm. body weight were used. Gastrointestinal ulcers were produced according to a modification of the method of Hay, *et al.* (7). The animals received daily a subcutaneous injection of 2 mg./Kg. Benadryl® followed by an intramuscular injection (into the back musculature) of 25 mg./Kg. histamine diphosphate and 5 mg./Kg. Benadryl® in beeswax and oil. Aqueous suspensions of the antacids were given by intraesophageal administration in two doses; in the morning and in the evening. Carbonic anhydrase inhibitors were given subcutaneously preceding each injection of histamine and additional quantities were incorporated into the beeswax and oil containing the histamine. All survivors were autopsied on the 10th day and the stomach and duodenum examined for ulceration. Animals which died during the experimental period were autopsied as soon after death as possible. Degree of ulceration (0, 1 to 4 plus) was established and the index of ulceration calculated according to the arbitrary standards and formula of Pauls, *et al.* (13).

According to this formula:

$$\text{Index of ulceration} = \frac{\text{Mean degree of ulceration} \times \% \text{ of group ulcerated}}{100}$$

The drugs were incorporated into beeswax and oil in the following manner: Histamine diphosphate powder was kept in a desiccator prior to use for at least twenty-four hours. It was triturated in a preheated mortar until it became homogeneous. Then the amount desired was weighed into a second heated mortar and melted beeswax was gradually added. This mixture was triturated until homogeneous, then hot mineral oil was added and again triturated. Antihistamine or carbonic anhydrase inhibitors if incorporated were treated similar to histamine. One daily dose of all drugs was incorporated into about 0.1 ml. of the beeswax-mineral oil mixtures. The ratio of beeswax to oil was about 1:3.4 ml. This mixture while molten can be drawn into a preheated tuberculin syringe and can be injected through a 20 gauge needle. Upon injection it solidifies and forms a depot, the absorption of which takes about twenty-four hours. This procedure has to be followed exactly, if the histamine contains water, or is not homogeneously distributed, ulcers will not be produced. The ratio of beeswax and oil must be established for each lot of material and the "depot histamine" tested for rate of absorption in preliminary studies preceding each experiment.

Antihistamines were used as a protective mechanism, since they have been shown not to influence histamine-induced gastrointestinal ulceration (4–6) yet to protect against the toxic effects of histamine. Carbonic anhydrase inhibitors were given subcutaneously as well as in the depot, in order to produce uni-

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[1] Our thanks are due to Dr. R. O. Roblin, Jr. of the American Cyanamid Co. for supplying this material.

[2] We are greatly indebted to Dr. Richard H. Barnes of Sharp & Dohme for providing us with Dirnate.

TABLE I.—DOSE LEVELS OF VARIOUS DRUGS AND RESULTS

Daily Treatment	No. of Animals with Ulcers/ Total No. of Animals	% of Animals with Ulcers	Mean Degree of Ulceration	Index of Ulceration
25 mg./Kg. depot-histamine and 5 mg./Kg. Benadryl i.m.; 2 mg./Kg. Benadryl s.c.	17/21	83.9	2.62	2.12
DASC 2 × 0.05 Gm. p. os.	6/10	60.0	0.90	0.54
DASC 2 × 0.5 Gm. p. os.	3/9	33.3	0.66	0.22
Dihydroxy aluminum amino acetate 2 × 0.05 Gm. p. os.	5/10	50.0	1.30	0.65
$CaCO_3$ 2 × 0.05 Gm. p. os.	7/10	70.0	1.5	1.05
$CaCO_3$ 2 × 0.5 Gm. p. os.	7/9	77.7	2.0	1.55
$NaHCO_3$ 2 × 0.05 Gm. p.os.	9/10	90.0	1.90	1.71
Diamox® 0.02 Gm./Kg. i.m. depot and 0.02 Gm. s.c.	7/9	77.7	2.71	2.11
Dirnate® 0.02 Gm./Kg. i.m. depot and 0.02 Gm./Kg. s.c.	7/9	77.7	2.70	2.10

form action for twenty-four hours. Single subcutaneous doses of these compounds are effective only for about four to six hours.

RESULT AND DISCUSSION

The dose levels used of the various drugs and the results obtained are shown in Table I. It appears that the carbonic anhydrase inhibitors Diamox® and Dirnate® did not influence gastrointestinal ulceration. Sodium bicarbonate had only a slight beneficial effect. Calcium carbonate was more effective, but the higher dose was slightly less effective than the lower dose. Dihydroxy aluminum sodium carbonate (DASC) as well as dihydroxy aluminum aminoacetate were highly effective in the low dose range. The high dose of DASC was the most effective of all drugs used in this study.

It is recognized by the authors that the pathomechanism of this type of gastrointestinal ulceration may still be different from many types of clinical ulceration. This study is therefore preliminary and further studies should be planned to select methods more closely approaching clinical situations and most suitable for large scale screening of antiulcer agents.

SUMMARY

1. Gastrointestinal ulcers were produced in guinea pigs by the intramuscular injection of depot-histamine-antihistamine mixtures.

2. The carbonic anhydrase inhibitors Diamox and Dirnate® given subcutaneously and in intramuscular depot did not inhibit ulceration.

3. Antacids given orally, twice daily, were effective in the following order of decreasing activity: DASC 0.5 Gm. > DASC 0.05 Gm. and dihydroxy aluminum aminoacetate 0.05 Gm. > $CaCO_3$ 0.5 Gm. and 0.05 Gm. > $NaHCO_3$ 0.05 Gm.

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A Note on the Aqueous Solubilities of Dibenamine® and Dibenzyline® Hydrochlorides*

By STEWART C. HARVEY and MARK NICKERSON†

IN CONNECTION with a study of the transformation and chemical reactions of the adrenergic blocking agents Dibenamine® (*N*,*N*-dibenzyl-β-chloroethylamine) and Dibenzyline® (*N*-benzyl-*N*-phenoxyisopropyl-β-chloroethylamine) and of related compounds (1, 2) it became necessary to investigate their aqueous solubilities and to estimate the minimal concentration of ethanol in water necessary to promote solution of the drugs. The solubilities of Dibenamine® and Dibenzyline® hydrochlorides and the methods employed for their determination may be of interest to future investigators.

The aqueous solubilities of both drugs were too low to be determined by several of the usual methods. Consequently, the two following methods were devised to permit an approximation thereof. In the first method, called the Turbidometric Method I, sufficient quantities of glass-distilled water were added to varying volumes of an agitated ethanolic solution of 0.0600 mg./ml. of Dibenamine® hydrochloride or Dibenzyline® hydrochloride to make a total volume of 5.00 ml. The resulting turbidity was determined immediately with a Klett-Summerson photoelectric colorimeter. The turbidity reading was plotted against the final concentration of drug; the relationship was essentially rectilinear, as shown in Fig. 1. The solubility was estimated by extrapolation to zero turbidity, and the intercept was taken to be the amount of drug remaining in solution. The concentration of ethanol in the medium at the points of intercept was 0.01–0.02%, so that the solubility expressed may be considered to be that in water alone. By this method the solubilities for Dibenamine® and Dibenzyline® hydrochlorides were approximately 5×10^{-5} and 1×10^{-4} mg./ml., respectively.

The second method, called the Visual Method II, consisted of the addition, to 1.0 ml. of an ethanolic solution containing varying concentrations of the drug, of enough water to produce a just-visible turbidity. The log of the final concentration of the drug in the mixture was plotted against the ratio of ethanol:water in the final mixture, and a straight line was obtained. The solubility was estimated by extrapolation to the drug-concentration axis, i. e., to the point where ethanol:water was zero (Fig. 2). By this method the estimated solubilities of the hydrochlorides of Dibenamine® and Dibenzyline® were 2×10^{-5} and 2.5×10^{-4} mg./ml., respectively. The values obtained by either method are not presumed to be exact, but they serve to establish values for the range and upper limit of the aqueous solubilities of these two drugs. It is also noteworthy that beyond approximately 66 volumes-per cent of ethanol for Dibenamine® hydrochloride and 69 volumes-per cent for Dibenzyline® hydrochloride a sharp increase in drug solubility occurs, such that quite high concentrations of drug may be obtained in solutions containing concentrations of ethanol greater than these.

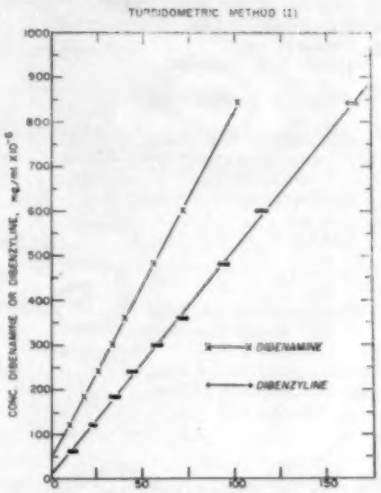

Fig. 1.—Turbidometer Reading

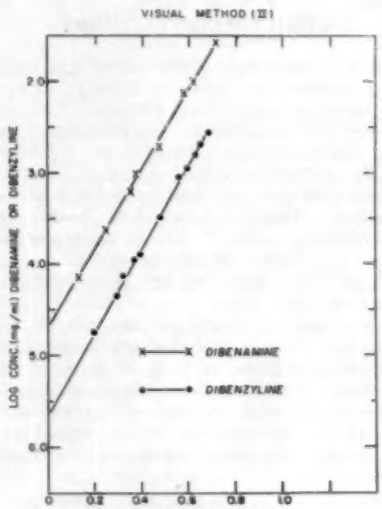

Fig. 2.—Ratio EtOH:H₂O

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This investigation was supported by grants-in-aid of research from the Smith, Kline & French Laboratories and from Givaudan-Delawana, Inc.
The authors are indebted to Dr. William S. Gump, Givaudan-Delawana, Inc., for the Dibenamine® and to Dr. Glenn E. Ullyot, Smith, Kline & French Laboratories for the Dibenzyline® used in this investigation.

A Note on the Preparation of a Cyclic Acetal from Chloral and Glucuronolactone*

BY FLOYD E. ANDERSON and ERNESTINE GELBLUM

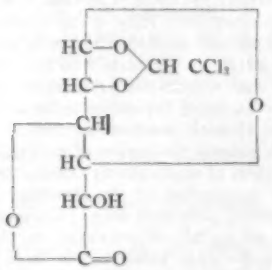
FOLLOWING the commercial availability of pure synthetic glucuronolactone (1) it became of interest to prepare and examine a number of derivatives of this biochemically important compound for their medicinal value. Reported here is a product of the interaction of glucuronolactone and chloral.

Chloral hydrate is regarded as a valuable sedative and hypnotic (2). However, numerous chemical derivatives of chloral have been made in attempts to improve its action, to eliminate its bitter, caustic taste and to diminish its irritating effect on the alimentary tract (3, 4).

Sedative and hypnotic properties have been noted in cyclic acetals derived from chloral and such polyols as glycerol (5) and glucose (6).

The preparation of a cyclic acetal from chloral and glucuronolactone was thought to be a desirable objective since such a compound would have within itself the means of its own detoxification. The assumption was, of course, that it would be metabolized hydrolytically to the glucuronide, a hemiacetal. It is a well-established fact that chloral is detoxified in man by reduction to the corresponding alcohol and subsequent elimination in the urine as the glucuronide (7).

Efforts to cause glucuronolactone and chloral to interact by azeotropic distillation (8) were unsuccessful. Equimolar amounts of the interactants when treated with cold concentrated sulfuric acid (9) produced a compound identical with that of Drefahl and Matschke (10). These investigators prepared their compound by the oxidation of β-chloralose with nitrogen dioxide and established the structure to be the 1,2-trichloroethylidene derivative (I) of glucuronolactone. A record of its having been tested for sedative and hypnotic effects has not hitherto appeared in the literature.



(I)

* Received November 18, 1954, from the Chemical Research Dept. of the Nepera Chemical Co., Yonkers, N. Y.

The compound does not appear to possess useful sedative or hypnotic properties.[1] In mice the oral LD_{50} dose is 540 mg./Kg. Delayed deaths appear at dose levels of 200 mg./Kg. When tested *in vitro* for its inhibitory properties on six representative pathogenic fungi, it was shown to be essentially inactive.

PREPARATION

A mixture of 30 cc. of concentrated sulfuric acid and 36.8 Gm. (0.25 mole) of anhydrous chloral was cooled and maintained at −5°. Glucuronolactone (44.0 Gm.; 0.25 mole) was added in small portions with stirring. The pasty mass was stirred intermittently over a four-hour period; it was then slowly poured with rapid stirring into a mixture of 300 Gm. ice and 100 cc. water. The impure precipitate when filtered and dried weighed 22.0 Gm. After recrystallization from distilled water three times, the white crystalline product weighed 15.0 Gm., m. p. 184–186°; $[\alpha]_D^{25}$ +30.5° (c 5.0, pyridine).[2]

Anal.—Calcd. for $C_9H_7Cl_3O_6$: C, 31.46; H, 2.24; Cl, 34.82. Found: C, 31.67; H, 2.52; Cl, 34.91.

SUMMARY

The cyclic acetal resulting from the interaction of chloral and glucuronolactone has been prepared and identified.

Pharmacological screening has shown the absence of useful sedative or hypnotic activity. The compound appears to have no antifungal activity.

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[1] The authors wish to acknowledge their indebtedness to Dr. R. J. Schachter for the pharmacological data and to Dr. Fred Barkley for the microbiological examination.

[2] Drefahl & Matschke (ref. 10) reported a m. p. 187° and $[\alpha]_D^{18}$ +31.3° (c 2.0, pyridine).

Book Notices

The Kinetic Basis of Molecular Biology. By FRANK H. JOHNSON, HENRY EYRING, and MILTON J. POLISSAR. John Wiley & Sons, Inc., New York (Chapman & Hall, Ltd., London), 1954. 874 pp. 16 x 24 cm. Price $15.

This is the first book in which modern reaction rate theory, developed since 1935, is extensively and systematically applied to the interpretation of biological reactions. The authors have used mathematics as needed to present the material in accordance with the basic laws governing the behavior of atoms, molecules, and ions. They review the essentials of themodynamics, and of classical, quantum and statistical mechanics; and the calculation of a four-atom reaction from first principles is illustrated. The text includes many illustrations, and an extensive bibliography and author index and a subject index are appended. This will undoubtedly be a valuable reference for those interested in this phase of biology.

Reports on the Progress of Applied Chemistry. Vol. 38. SOCIETY OF CHEMICAL INDUSTRY. Interscience Publishers, Inc., New York, 1953. 989 pp. 14 x 22 cm. Price $6.50.

As indicated by the title, this book outlines the progress of applied chemistry during 1953. It is divided into seven principal divisions under the following titles: Fuel and Fuel Products; Organic Chemistry; Inorganic Chemistry; Biological Products; Textiles, Plastics, Adhesives and Paints; Food and Agriculture; and Chemical Engineering and Water. Each main division is divided into several chapters, each contributed by authorities in the fields covered. The sections of principal pharmaceutical interest are those devoted to fine chemicals and medicinal substances; cosmetics and toilet preparations; fats, fatty oils and detergents; plastics; essential oils; general microbiological processes; and enzymes. References to the original literature upon which the various reports are based appear at the end of each chapter. Thus, the book serves as a useful reference in the compilation of bibliographies on the subjects covered.

Einführung in die Biologische Registriertechnik. By Dr. Med. HERBERT KLENSCH. Georg Thieme Verlag, Stuttgart, 1954 (Intercontinental Medical Book Corporation, New York 16, N. Y.). x + 222 pp. 24.75 x 17.75 cm. Price $4.85.

This book is intended to be an introduction to the field of biological registration. As it owes its existence to courses given by the author, it is arranged progressively in accordance with the complexity of the apparatus and the difficulty of operation. Dr. Klensch's small volume does not propose to compete with the large standard works in the field like Abderhalden or Bethe-Bergmann, but has the advantage, due to its small format, of being reasonably up to date. The presentation is clear, uncomplicated by mathematical formulations, and illustrated by drawings which are practically self-explanatory. The almost complete neglect of the non-German literature in the voluminous bibliography (over 400 items) was a matter of very considerable surprise to us.

Somministrazione dei Farmaci per Via Rettale: Tecnica de Preparazione delle Forme Farmaceutiche. By G. GIACOMINI and E. MASCITELLI. Gitti Editore, Milan, 1954. 341 pp. 24 x 17 cm. Price L 3500.

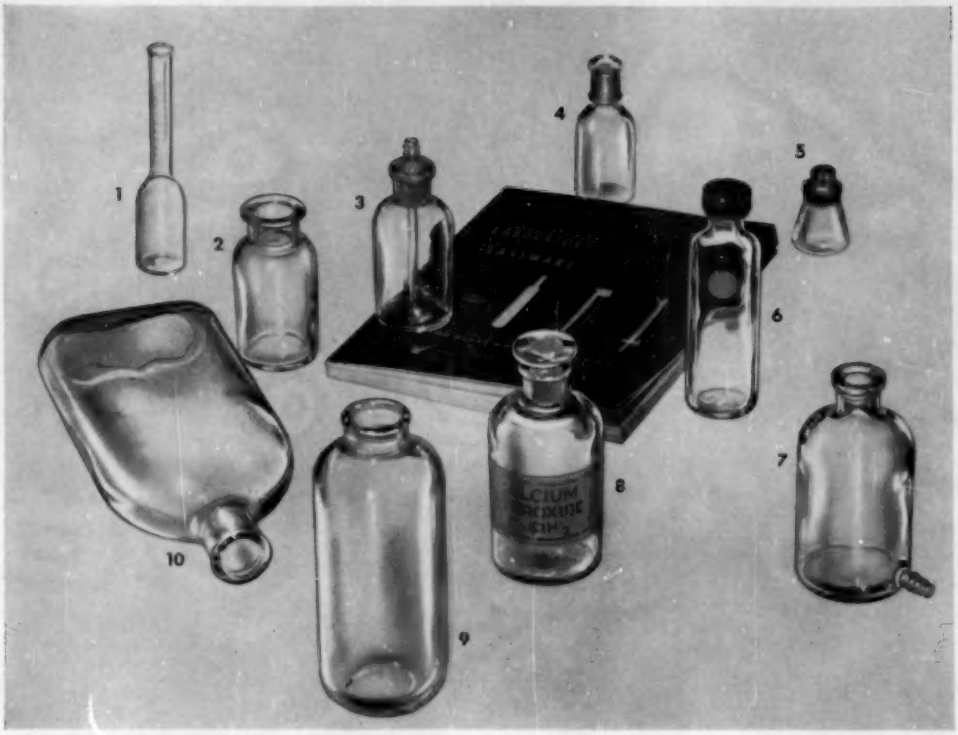
This book, which discusses the administration of pharmaceuticals by the rectal route or, in other words, suppositories, fills a definite gap in pharmaceutical literature. No comprehensive modern treatise on this subject seems to be available in the English or American literature. The first part of the work is given over to the anatomical background, the problem of absorption and the therapeutic indications arranged according to organs concerned. The second part takes up excipients in general, animal experimentation, gelatine, glycerin, preservatives, and, in great detail, the techniques used in the preparation of suppositories. Especially valuable is a long chapter (over 50 pages) on preparation of uncommon medicines for rectal administration. The bibliography contains references up to, and including, 1953. Highly recommended.

Organic Reactions. Vol. 8. ROGER ADAMS, Editor-in-Chief. John Wiley & Sons, Inc., New York, 1954. viii + 437 pp. 15.5 x 23.5 cm. Price $12.

This is the eighth volume of a series on organic reactions, the first of which appeared in 1932. The aim of the series has been to provide a compilation of all important synthetic chemical reactions, and this purpose has been fully accomplished. In the current volume, as in the earlier ones, each chapter has been written by chemists who have had special experience with the particular reaction discussed.

In each chapter of the entire series a comprehensive survey of each reaction covered is presented, including a general discussion of method, modifications, examples of applicability, special precautions, a detailed description of the procedure, expected yields, and other pertinent data. Compounds which have been prepared, or subjected to the reaction under discussion, are tabulated in each chapter.

The reactions covered in this volume are indicated by the chapter headings: A Catalytic Hydrogenation of Esters to Alcohols; The Synthesis of Ketones from Acid Halides and Organometallic Compounds of Magnesium, Zinc, and Cadmium; The Acylation of Ketones to Form β-Diketones or β-Keto Aldehydes; The Sommelet Reaction; The Synthesis of Aldehydes from Carboxylic Acids; The Metalation Reaction with Organolithium Compounds; β-Lactones; and The Reaction of Diazomethane and its Derivatives with Aldehydes. Adequate references to the original literature are cited and the book is provided with an excellent subject index.



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5. 1620–Specific Gravity, Hubbard-Carmick
6. 635–Milk Dilution, Graduated, With Screw Cap
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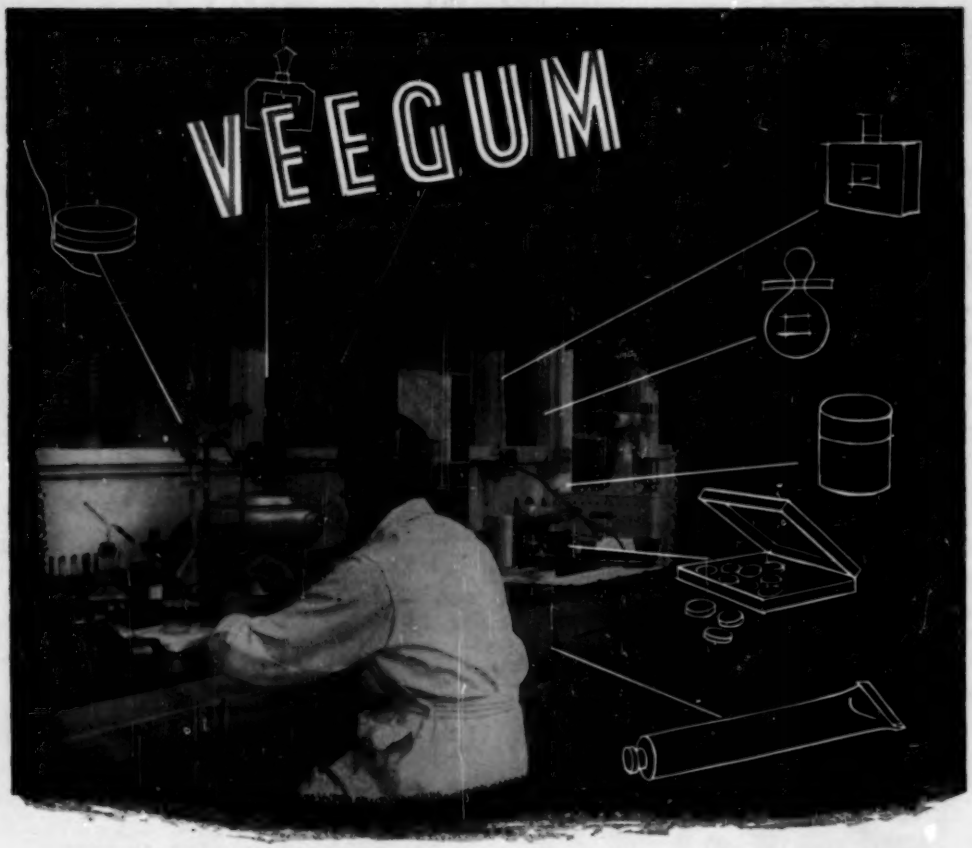

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